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## Effects of MDL-72222 on cocaine- and morphine-induced conditioned place preference in preweanling rats

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EFFECTS OF MDL-72222 ON COCAINE- AND MORPHINE-INDUCED  
CONDITIONED PLACE PREFERENCE IN PREWEANLING RATS.

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A Thesis  
Presented to the  
Faculty of  
California State University,  
San Bernardino

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In Partial Fulfillment  
of the Requirement for the Degree  
Master of Arts  
in  
Psychology

---

by  
Bonnie Sue Butterfield

June 1997

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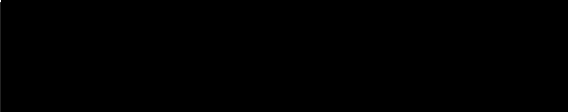
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by

Bonnie Sue Butterfield

June 1997

Approved by:

  
Sanders A. McDougall, Chair, Psychology

6/6/97  
Date

  
Stuart R. Ellins

  
Cynthia Crawford

## ABSTRACT

In adult rats, antagonism of the 5-HT<sub>3</sub> serotonin receptor blocks the acquisition of a morphine-induced conditioned place preference (CPP). In the present study, the ability of the selective 5-HT<sub>3</sub> serotonin receptor antagonist MDL-72222 to modulate the acquisition and expression of cocaine- and morphine-induced CPP was assessed in 17-day-old rats. An abbreviated CPP procedure was used in which rats received two saline-odor (lemon or almond) pairings on the first conditioning day, and two saline-, cocaine-, or morphine-odor pairings on the second day. Preference was assessed on the third day. To determine the effects of 5-HT<sub>3</sub> receptor antagonism on the acquisition of CPP, rats were given MDL-72222 (0.5-4.5 mg/kg, IP) 30 min prior to being conditioned with cocaine (20.0 mg/kg, IP) or morphine (0.5 mg/kg, IP). To determine the effects of 5-HT<sub>3</sub> receptor antagonism on the expression of CPP, rats were injected with MDL-72222 30 min prior to preference testing. As expected, both cocaine and morphine produced robust place preference conditioning in preweanling rats. Surprisingly, MDL-72222 did not affect the acquisition or expression of cocaine- or morphine-induced CPP. These results suggest that the 5-HT<sub>3</sub> serotonin receptor system may differentially modulate DA- and opioid-mediated reward across ontogeny. The known peak surge in the density of certain dopamine



receptor subtypes within the mesolimbic system coincide with the preweanling stage of development. An animal's ability to acquire and express memories and behaviors related to rewarding experiences depends upon the robust functioning of the reward center. The mastery required to survive the weaning period may depend upon a temporary alteration in neurotransmission that reduces the risk of lowering reward functioning.

## ACKNOWLEDGEMENTS

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## TABLE OF CONTENTS

ABSTRACT . . . . .	iii
ACKNOWLEDGEMENTS . . . . .	v
LISTS OF ILLUSTRATIONS . . . . .	ix
OVERVIEW . . . . .	1
INTRODUCTION . . . . .	5
BASIC STRUCTURE AND FUNCTION OF DA PATHWAYS . . . . .	6
Nigrostriatal Pathway . . . . .	7
Mesocortical Pathway . . . . .	9
Mesolimbic Pathway . . . . .	10
DA Receptors . . . . .	11
Opioid Structures Within The Mesolimbic system . . . . .	14
PHARMACOLOGY OF REWARD . . . . .	17
DA REWARD . . . . .	18
Instrumental Conditioning Studies . . . . .	18
Operant Conditioning Studies . . . . .	20
DA Agonists and Operant Conditioning . . . . .	22
CPP . . . . .	24
OPIOID REWARD . . . . .	31
Operant Conditioning Studies . . . . .	31
CPP . . . . .	33

SEROTONIN STRUCTURE AND FUNCTION .....	34
Serotonin Pathways .....	34
Serotonin Receptors: Distribution And Function .....	38
DA AND SEROTONIN INTERACTIONS .....	43
DA and Serotonin Interaction: Receptors .....	45
DA and Serotonin Interaction: Rat Behavior .....	53
DEVELOPMENTAL IMPLICATIONS .....	56
Ontogeny of Neurotransmitter Systems: DA .....	56
Ontogeny of Neurotransmitter Systems: Opiates .....	57
Ontogeny of Neurotransmitter Systems: Serotonin .....	59
GENERAL PURPOSE AND HYPOTHESIS .....	61
EXPERIMENT 1 .....	64
Method .....	64
Subjects .....	64
Apparatus .....	64
Procedure and Drugs .....	65
Data Analysis .....	66
Research Setting .....	66
Results .....	66
Locomotor Activity .....	66
Conditioned Place Preference .....	66

Summary .....	71
EXPERIMENT 2 .....	75
Method .....	75
Subjects .....	75
Procedure And Drugs .....	75
Results .....	77
Locomotor Activity .....	77
Conditioned Place Preference .....	77
Summary .....	83
DISCUSSION .....	85
CONCLUSION .....	96
REFERENCES .....	99

## LIST OF ILLUSTRATIONS

	Page
Figure 1. Dopamine Pathways: Coronal and Lateral.....	8
Figure 2. Mesolimbic Dopamine System.....	12
Figure 3. Dopamine Receptor Model.....	13
Figure 4. Morphine increases DA release in the NA indirectly through its attenuation of GABA release.....	16
Figure 5. Blockade Of CPP Acquisition Or Expression.....	29
Figure 6. Serotonin Pathways: Coronal Section.....	36
Figure 7. Serotonin Pathways: Lateral View.....	37
Figure 8. Mesolimbic Dopamine System Showing Various Receptor Locations.....	39
Figure 9. Serotonin Receptor Model.....	40
Figure 10. Serotonin 5-HT <sub>3</sub> Receptors Gate Ionic Channels.....	47
Figure 11. Model Of 5-HT <sub>3</sub> Modulation Of DA Reuptake.....	47
Figure 12. Two Different Molecular Proteins That May Transport DA.....	49
Figure 13. Model of Potential Interactions Between A GABA Interneuron and Serotonin and Dopamine Neurons.....	50
Figure 14. Protocol Design of Expression Experiment.....	67

Figure 15.	Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each).....	68
Figure 16.	Mean time that rats spent (seconds) in the morphine-paired chamber (+SEM) of the CPP apparatus on test day.....	69
Figure 17.	Mean time that rats spent (seconds) in the cocaine-paired chamber (+SEM) of the CPP apparatus on test day.....	70
Figure 18.	Mean time spent (seconds) in the saline-paired chamber (+SEM) of the CPP apparatus on test day.....	72
Figure 19.	Mean time spent (seconds) in the preferred chamber (+SEM) by saline- or morphine-treated rat pups on test day.....	73
Figure 20.	Mean time spent (seconds) in the preferred chamber (+SEM) by saline- or cocaine-treated rat pups on test day.....	74
Figure 21.	Protocol Design of Acquisition Experiment.....	76
Figure 22.	Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each).....	78

Figure 23.	Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each).....	79
Figure 24.	Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each).....	80
Figure 25.	Mean time that rats spent (seconds) in the morphine-paired chamber (+SEM) of the CPP apparatus on test day.....	81
Figure 26.	Mean time that rats spent (seconds) in the cocaine-paired chamber (+SEM) of the CPP apparatus on test day.....	82
Figure 27.	Mean time that rats spent (seconds) in the saline-paired chamber (+SEM) of the CPP apparatus on test day.....	84
Figure 28.	Development of D <sub>1</sub> and D <sub>2</sub> receptor sites through equilibrium saturation studies of D <sub>1</sub> ( <sup>3</sup> H-SCH 23390) and D <sub>2</sub> ( <sup>3</sup> H]-(-) sulpiride) binding at increasing ages in rat brain development.....	91
Figure 29.	Mean regional density of D <sub>1</sub> receptors during development of the 15-day-old to 21-day-old.....	92
Figure 30.	Dopamine receptors D <sub>1</sub> and D <sub>2</sub> in rat brain.....	94



## OVERVIEW

Science is revealing that a homeostasis exists within an individual's normal brain chemistry. Disruption of the functional stability between the levels of various neurochemicals can lead to temporary or permanent disease. No area of the brain is more important to an individual's psychological health than the mesolimbic system (Davis, Kahn, Ko, & Davidson, 1991). This system has been implicated in the sense of mental well being and in the reinforcement properties of drugs of addiction (Nathan, Dominique, Schatzberg, & Nemeroff, 1995; Wise & Bozarth, 1987).

Evidence from several experimental paradigms have implicated the dopamine (DA) pathway in an endogenous system of reward (Ettenberg & Camp, 1986; Olds, 1978). Instrumental conditioning, operant conditioning and conditioned place preference (CPP) studies have suggested that DA neurotransmission is essential for the establishment of the natural reinforcement properties of satiation, as well as the addictive qualities of substances of abuse (Beninger et al., 1987; Fibiger & Phillips, 1982; Wise & Bozarth, 1987; Wise, Spindler, & Legault, 1978). Additionally, both the acquisition and expression of conditioned behaviors are shown to be mediated by separate DA receptor

mechanisms (Hiroi & White, 1989, 1990; Spyraiki, Fibiger, & Phillips, 1982a; White & Carr, 1984, 1985).

Through manipulations of the specific neurochemicals that are involved in reinforcement, the reward pathway has been shown to be stimulated by drugs that activate DA neurons, causing DA release into the nucleus accumbens (NA) (deWit & Wise, 1978; DiChiara & Imperato, 1988; DiChiara & North, 1992; Grant, 1995; Gysling & Wang, 1983; Shippenberg, Bals-Kubik, & Herz, 1993; Wise & Bozarth, 1987). Addictive drugs that excite the DA reward system, such as morphine and cocaine, allow the acquisition and expression of reward; whereas, neurochemicals that antagonize DA neurotransmission appear capable of suppressing reward (Calcagnetti, Keck, Quartrella, & Schechter, 1995; DiChiara & North, 1992; Hoffman, 1989; Wise & Bozarth, 1987). The expression of reinforced behavior is believed related to the mnemonic aspects of reward, possibly through afferents coming into the NA from the amygdala (Hiroi & White, 1991).

Thus, DA maintains a central place of importance in the reward process (DiChiara & Imperato, 1988). In addition to the role played by mesolimbic DA neurons and their fiber pathways, evidence has implicated the existence of other neurotransmitters within the reward system (Costall, Naylor, Mardens, & Pycock, 1976; DiChiara & North,

1992). For example, both the serotonin and opioid systems modulate the DA reward pathway. These two neurotransmitter systems have been implicated in the normal functioning of reward mechanisms and may impact the effects that addictive drugs have on behavior (Costall et al., 1976; DiChiara & North, 1992; Imperato & Angelucci, 1989; Spanagel, Herz, & Shippenberg, 1992). Consequently, experimental research has begun to focus on the role played by serotonin in the reward process (Grant, 1995; Imperato & Angelucci, 1989). Studies of serotonin agonists and antagonists have provided evidence that the serotonin receptor is important for reward. Antagonism of the serotonin receptor subtype 5-HT<sub>3</sub> appears to abolish drug-induced reward (Joharchi, Sellars, & Higgins, 1993; Suzuki, Shiozaki, Masukawa, & Misawa, 1992). This suggests that serotonin regulates mesolimbic DA neurotransmission (Carboni, Acquas, Frau, & DiChiara, 1989; Imperato & Angelucci, 1989).

There are indications that a functioning reward system exists in preweanling rats, although some interesting age-dependent differences have been reported (McDougall, Nonneman, & Crawford, 1991). Evidence indicates that in addition to a fairly mature endogenous reward system, neonates also have the ability to respond to drugs with reinforcing properties. Morphine and cocaine enhance DA levels in preweanling rats, an effect that also enhances reinforced behaviors. Consequently,

blockade of DA receptors attenuates the reinforcing properties of drugs like morphine and cocaine (Barr & Rossi, 1992; Laviola, Dell'Omo, Alleva, & Bignami, 1992; Pruitt, Bolanos, & McDougall, 1995).

It also appears that preweanling rats have a fully developed serotonin system, as receptor binding studies demonstrate that serotonin receptors are in place and functioning shortly after birth (Daval et al., 1987; Laviola et al., 1992; Pranzatelli, 1992). However, little is known about the interaction between the DA and serotonin systems in the preweanling rat, so it is uncertain whether the serotonin system modulates reward functioning in these young animals. Additionally, it has never been determined what brain areas are responsible for serotonin's reward attenuation abilities or whether separate mechanisms control the acquisition and expression of reward. Therefore, the purpose of this project is: first, to determine whether serotonin 5-HT<sub>3</sub> receptor antagonists will block both the acquisition and expression of DA and opiate reward and, second, to determine what brain areas are critical for serotonin's reward suppressing effects

## INTRODUCTION

DA is enormously important to psychological well-being (Kapur & Remington, 1996). Pathology in DA pathways have been implicated in mood disorders (Nathan et al., 1995), the disordered thought and affect in schizophrenia (Davis et al., 1991), the involuntary tics and coprolalia of Tourette's disorder (Malison et al., 1995), the motor impairments of Parkinson's disease (Agid, 1991; Balk et al., 1995), the inattention and hyperactivity in children with attention-deficit hyperactivity disorder (ADHD) (Castellanos et al., 1994) and in the paranoid symptoms of Alzheimer's disease (Nazarali & Reynolds, 1992). DA levels within the mesolimbic DA reward pathway have also been directly implicated in the rewarding properties of drugs of abuse (Wise & Bozarth, 1987). The many devastating abnormalities in behavior that result from a disruption of DA neurotransmission indicates its immense importance to normal functioning.

Although the exact causes of schizophrenia, Alzheimer's Disease, Tourette's Disorder and ADHD remain unknown, many of the symptoms are alleviated by antagonism or agonism of DA system functioning. DA receptor antagonists, such as chlorpromazine and haloperidol, are used to alleviate the psychotic symptoms of schizophrenia and Alzheimer's disease (Davis et al., 1991; Nazarali & Reynolds, 1992). Symptoms of

Tourette's syndrome are also alleviated by DA antagonists (Malison et al., 1995). Methylphenidate, an indirect DA agonist related to amphetamine, is used successfully to treat many cases of ADHD (Castellanos et al., 1994).

Many addictive drugs activate the DA system. Research utilizing animal models of reward have implicated DA neurotransmission in the rewarding effects of drug addiction (Bozarth & Wise, 1981; Hoffman, 1989; Wise, 1983). For example, systemic or localized injections of addictive drugs, such as morphine, cocaine and amphetamine, increase DA levels within the mesolimbic region of the rat brain (DiChiara & Imperato, 1988; Wise & Bozarth, 1987). Conversely, antagonism of DA receptors inhibits DA release and attenuates drug-induced behaviors in rats (DiChiara & Imperato, 1988; Rompre & Wise, 1988; Yokel & Wise, 1976).

Thus, DA neurotransmission appears to have an important role in normal functioning. Diseases of the nervous system or drug-induced imbalances can cause disruptions in the DA pathways resulting in pathological behaviors.

### BASIC STRUCTURE AND FUNCTION OF DA PATHWAYS

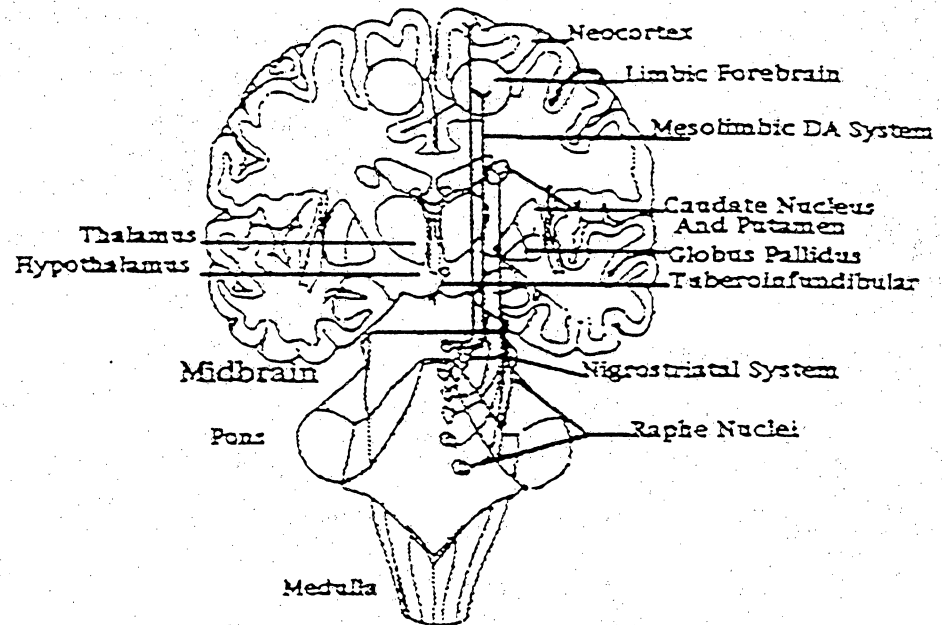
The DA system has a highly organized arrangement of projections that are classified into the nigrostriatal and mesolimbic-mesocortical

systems. The nigrostriatal system primarily extends from the substantia nigra to the striatum (i.e., caudate-putamen). Additional DA fibers project from the ventral tegmental area (VTA) to either the limbic system (to form mesolimbic connections) or to the cortex (to form mesocortical connections) (Grant, 1995; Kandel, Schwartz, & Jessell, 1995; Nicholls, Martin, & Wallace, 1992; Phillips, 1984; Routtenberg, 1978; Wise & Rompre, 1989). Although frequently considered together, the mesolimbic-mesocortical systems will be discussed separately to simplify discussion (see Figure 1).

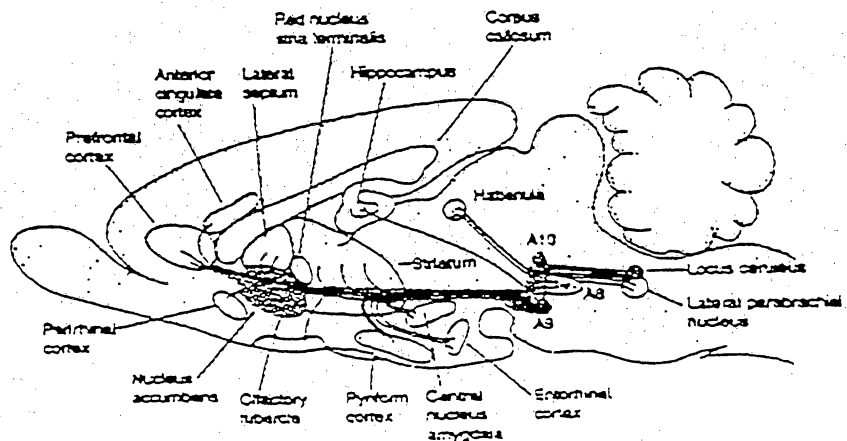
#### Nigrostriatal Pathway

The nigrostriatal system is formed by DA neurons that originate in the substantia nigra and project to several striatal sites, including the caudate-putamen, subthalamic nucleus and the globus pallidus. The rat substantia nigra has 7000 DA cells which contain over 250,000 processes extending into the caudate-putamen (Nicholls et al., 1992). When the neurons are activated, DA is released at the terminals and mediates the voluntary control of movement (Kandel et al., 1995). Parkinson's disease, which is characterized by muscle rigidity, tremor and difficulty in initiating movement, occurs when the DA cells of the nigrostriatal pathway degenerate (Agid, 1991).

# Dopamine Pathways-Coronal Section



## Lateral View



Mesostriatal System located in A3 and A9  
Mesolimbic System located in A10

(Adapted from Kandel et al., 1995)

Figure 1. Dopamine Pathways: Coronal and Lateral.



### Mesocortical Pathway

The DA cells of the mesocortical system primarily have their origin in the ventral tegmentum (Area 10). Their projections extend to the cerebral cortex where they are thought to contribute to cognition (Kandel et al., 1995; LeDoux, Iwata, Cicchetti, & Reis, 1988). DA fiber projections are most dense in the prefrontal cortex, where the functions of motivation and planning are believed to occur (Fibiger & Phillips, 1986, 1988; Goeders & Smith, 1983).

The temporal organization of behavior, attention and social responses may also be modulated by activity in this pathway (Castellanos et al., 1994). Much research indicates that an interaction exists between the cortical and subcortical DA systems, through fiber connections traversing both regions. A reduction in prefrontal DA innervation causes increased activity in subcortical brain areas (Deutch, Clark, & Roth, 1990; Gariano & Groves, 1988; Kandel et al., 1995; Nicholls, 1992). Afferents from the prefrontal cortex are believed to make synaptic connections with dopaminergic structures in the VTA (Deutch et al., 1990). Moreover, prefrontal afferent fibers are in close proximity to tyrosine hydroxylase positive terminals within the mesolimbic pathway (Sesack & Pickel, 1992). This suggests that the cortex and the mesolimbic system do not function in isolation from one another: Drugs that impact

one may exert changes on the other.

Furthermore, it has also been demonstrated that activation of the prefrontal cortex directly increases dopaminergic activity within non-cortical DA pathways (Gariano & Groves, 1988; Murase, Mathe, Grenhoff, & Svensson, 1993; Taber & Fibiger, 1993). In contrast, input going from the dopaminergic system to the prefrontal cortex appears to be inhibitory (Jaskiw, Weinberger, & Crawly, 1991; Louilot, LeMoal, & Simon, 1989). A reduction in prefrontal dopaminergic activity results in increased activity and/or heightened sensitivity within the mesolimbic region (Deutch et al., 1990; Haroutunian, Kanof, & Davis, 1989; Pycock, Kerwin, & Carter, 1980; Rosin, Clark, Goldstein, Roth, & Deutch, 1992).

#### Mesolimbic Pathway

The mesolimbic system is critically involved in reward. This system is composed of DA cell bodies that are found in the VTA and the axons of these DA neurons extend into the NA and amygdala. Therefore, activation of cell bodies in the VTA results in increased DA release from terminal fibers in the NA and the amygdala (DiChiara & Imperato, 1988; Hiroi & White, 1991b; Phillips, 1984; Routtenberg, 1978; Wise & Rompre, 1989). The neural input into the VTA is from the medial forebrain bundle (MFB). When the MFB is stimulated, it excites non-dopaminergic fibers which synapse on DA neurons in the VTA and substantia nigra

(Shizgal & Murray, 1989) (see Figure 2).

### DA Receptors

Following its release from the DA terminal, DA neurotransmitter binds to specific receptor sites (see Figure 3). These receptors are classified according to similarities in genetic structure and the presence of common second messenger mechanisms. One family of DA receptors comprises the D<sub>1</sub> and D<sub>5</sub> subtypes. The D<sub>1</sub> receptors are found in the mesocortical system. The second family of DA receptors is called D<sub>2</sub> and also includes the D<sub>3</sub> and D<sub>4</sub> types.

The DA receptor prominent in the nigrostriatal system is the D<sub>2</sub> subtype, whereas D<sub>3</sub> and D<sub>4</sub> receptors are primarily distributed in the mesolimbic system. Presynaptic DA receptors appear to be localized in two main areas: one is on DA cell bodies in the mesolimbic region (called somatodendritic autoreceptors), where they modulate the firing of DA neurons; the second is on DA axonal terminals (called terminal autoreceptors), where they modulate release of DA (Cooper, Bloom, & Roth, 1991; deKeyser, 1993).

## MESOLIMBIC DOPAMINE SYSTEM

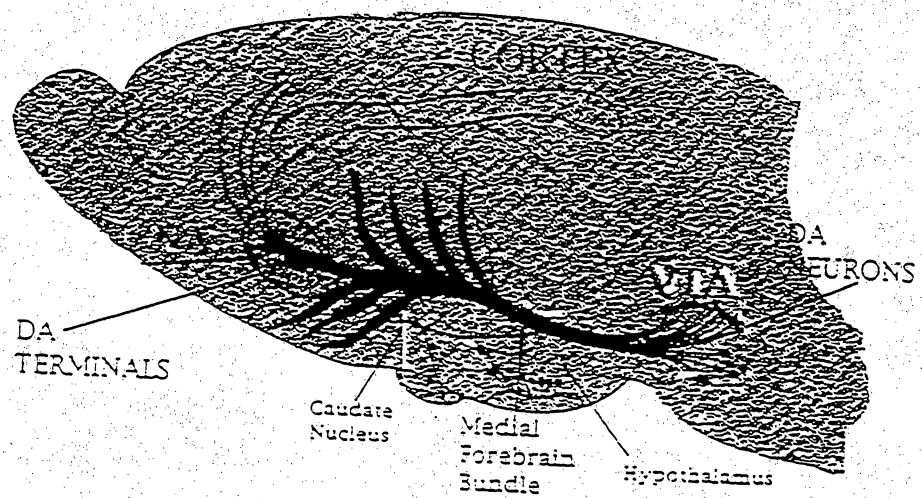
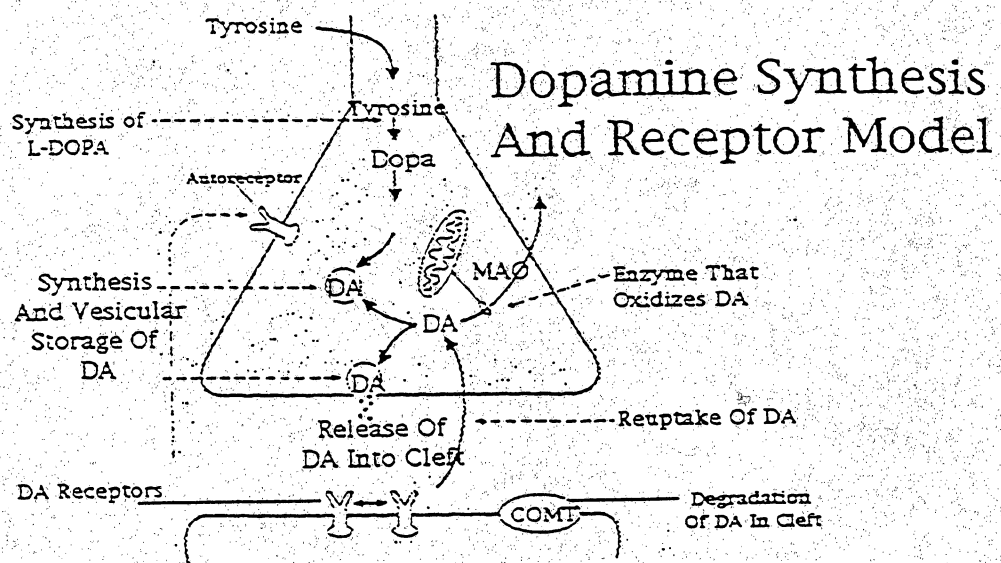


Figure 2 Mesolimbic Dopamine System.



(Adapted from Kandel et al., 1995)

Figure 3. Dopamine Receptor Model.

### Opioid Structures Within The Mesolimbic system

Opioid receptors and natural opioids, called enkephalins and endorphins, are found in abundance within the mesolimbic system (Kalin & Loevinger, 1983; Spanagel, 1995). Binding studies demonstrate that opioid and DA receptors are co-localized in the VTA and fibers containing enkephalin are found together with DA neurons (Manzanares, Lookingland, & Moore, 1991; Morel & Pelletier, 1986). Some of the opioid receptor subtypes have been implicated in the mediation of mesolimbic DA functioning (DiChiara & Imperato, 1988; Mansour, Khachaturian, Lewis, Akil, & Watson, 1988).

Opiate receptor binding sites and their corresponding neurotransmitters are divided into three major subtypes called mu ( $\mu$ ), delta ( $\delta$ ) and kappa ( $\kappa$ ) (Lord, Waterfield, Hughs, & Kosterlitz, 1977). Morphine and met-enkephalin have a high affinity for  $\mu$  receptors, whereas leu-enkephalin preferentially binds to  $\delta$  receptors. D-ala-met-enkephalin, a specific  $\mu$  receptor agonist is found to bind directly to endogenous opiate met-enkephalin  $\mu$  receptors localized in the VTA (Sato & Minami, 1995). However, mRNA studies have found evidence for the presence of  $\mu$  receptors in both the VTA and the NA (Azaryan, Coughlin, Buzas, & Cox, 1996). High concentrations of the endogenous  $\delta$  neurotransmitter leu-enkephalin and  $\delta$  receptors are also found in the

NA and the VTA (Mansour et al., 1988; Satoh & Minami, 1995).  $\kappa$  receptor sites are located within many brain regions including the NA, VTA, medial prefrontal cortex and lateral hypothalamus (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993).  $\kappa$  receptors are bound by deltaporphins and research suggests that they are important for mediating aversive stimulation (Erspamer et al., 1989).

When  $\mu$  receptors are bound by an agonist, they activate DA neurons in the mesolimbic pathway (see Figure 4). Specifically, activation of those  $\mu$  receptors located on GABAergic interneurons in the VTA inhibits the release of GABA (Brady, Herkenham, Long, & Rothman, 1989; La Motte, Snowman, Pert, & Snyder, 1978).

These GABA neurons normally inhibit the DA fibers of the mesolimbic pathway, so a lack of GABA activation prevents the inhibition of DA neurons and, indirectly, results in DA activation (DiChiara & North, 1992; Klein et al., 1993). Microdialysis studies indicate that when opiates bind to  $\mu$  receptors, an activation of mesolimbic DA neurons occurs (DiChiara & Imperato, 1988). Specifically, when morphine is injected exclusively into the VTA, DA neurons are activated causing increases in DA levels within the NA (DiChiara & Imperato, 1988).

Figure 3

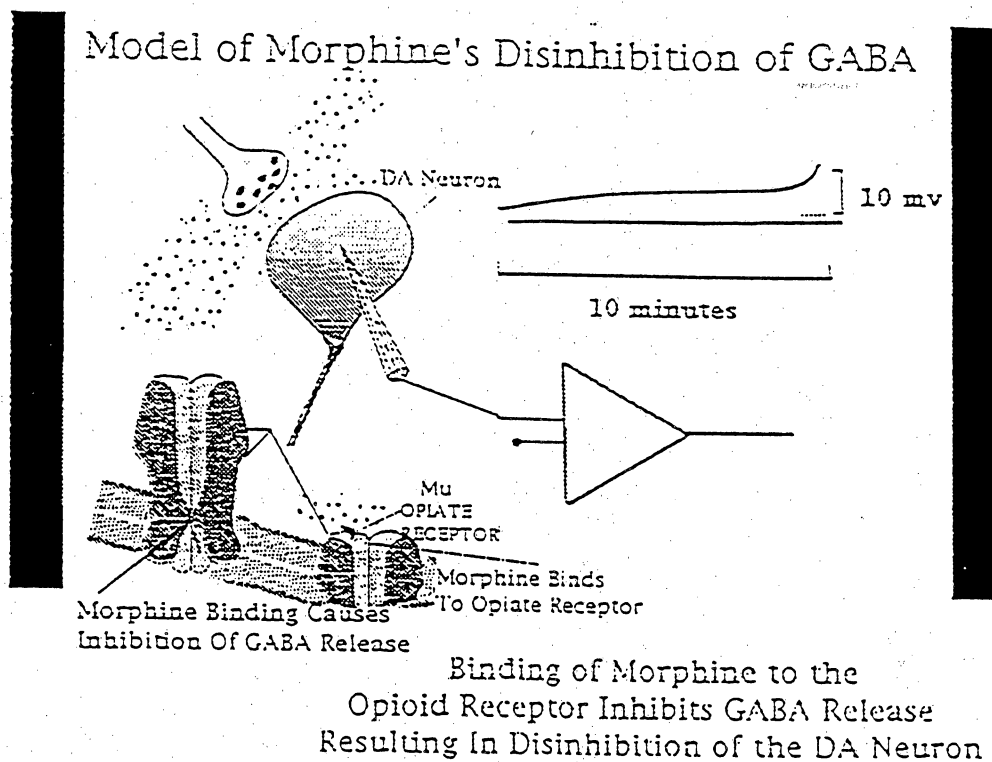


Figure 4. Morphine increases DA release in the NA indirectly through its attenuation of GABA release.



Morphine enhances DA activity if given either systemically or locally injected into the VTA, but not when morphine is injected directly into the NA. A model describing the modulation of the mesolimbic system by two opposing opioid systems has been constructed by Spanagel et al. (1992). They propose that the DA neurons projecting to the NA can be disinhibited by  $\mu$ -opioid receptors located on those GABA inhibitory interneurons in the VTA (see Figure 4). The disinhibition of the DA neurons causes a measurable increase in NA DA release, which is reflected in behavior (Spanagel et al., 1992). Whereas, the activation of  $k$ -opioid receptors located in the NA results in the inhibition of DA release and has aversive and sedative effects on behavior (Spanagel et al., 1992).

In summary, the mesolimbic DA system, whose DA neurons arise in the VTA and extend their axons into the NA, has been implicated in the mediation of reward (Ettenberg, 1989; Wise, 1983; Wise & Rompre, 1989).  $\mu$  and  $\delta$  opioid receptors enhance DA function by disinhibiting the DA neurons of the mesolimbic pathway, whereas  $k$  receptors directly inhibit DA release in the NA (DiChiara & North, 1992; Klein et al., 1993; Spanagel et al., 1992).

## PHARMACOLOGY OF REWARD

Animal research suggests that the brain has natural reinforcement mechanisms, with both endogenous DA and opioid processes modulating

reward (Olds & Milner, 1954; Salamone, Cousins, McCullough, & Carrier, 1994; Shizgal & Murray, 1989; Wise, 1983). There is a large amount of evidence that the mesolimbic DA pathway is responsible for the reinforcing effects of natural rewards such as food and water. There is also considerable support for the role played by the DA pathway in the reinforcement properties of addictive drugs. The most important paradigms assessing reward are the instrumental conditioning, operant conditioning, and CPP paradigms (Ettenberg & Camp, 1986; Hoffman, 1989; Wise & Rompre, 1989).

## DA REWARD

### Instrumental Conditioning Studies

Utilizing the instrumental conditioning paradigm, several laboratories have investigated the reinforcing effects of food and water consumption by challenging DA receptors with antagonists (Ettenberg & Camp, 1986; Ettenberg & Horvitz, 1990; Ettenberg, Koob, & Bloom, 1981; Ljungberg & Ungerstedt, 1979). In a study that examined the role of DA in water reward, an experimental group received a pretreatment of either pimozide or haloperidol (DA receptor antagonists). Response times of untreated, water deprived animals was faster when compared to thirsty rats that had been pretreated with DA antagonists (Ettenberg & Camp, 1986; Ettenberg & Horvitz, 1990).

Researchers also used DA antagonists to challenge the reinforcing properties of food reward. Blockade of DA receptors by haloperidol reduced response times and running speeds of rats towards a food reward. Apparently, the reinforcement value of food pellets was effectively suppressed by DA antagonists, reducing the importance of the anticipated reward (Ettenberg & Camp, 1986). Thus, through DA antagonism, the reinforcement qualities of food and water reward could be attenuated in the runway paradigm (Ettenberg & Camp, 1986; Ettenberg & Horvitz, 1990).

Importantly, other researchers have contended that drug-induced motor deficits, rather than reward diminution, could account for the decrease in reinforced responding (Ettenberg, Koob, & Bloom, 1981; Ljungberg & Ungerstedt, 1979). To assess the possibility of a motor confound, experimenters either tested rats in a non-drugged state or utilized an extinction paradigm to confirm that DA antagonism does attenuate reinforced responding. For example, while testing water reward in a non-drugged state, Ettenberg and Horvitz (1990) demonstrated a reduced running speed in thirsty rats. Because the animals were evaluated 24 hours after DA antagonist treatment, it confirmed that the attenuation of the reinforced response was not related to drug-induced motor impairments (Ettenberg & Horvitz, 1990). Other experiments

utilized the extinction paradigm to verify DA attenuation of food reward. Rats treated intermittently with DA antagonists demonstrated resistance to extinction, despite the presence of continuous food reward (Ettenberg & Camp, 1986; Salamone, 1986). Therefore, when considered together these results suggest that DA antagonists block the reward value of food and water reinforcers.

Utilizing the instrumental conditioning paradigm, several researchers attempted to determine which DA receptor subtypes were responsible for natural reward (Fowler, Liou, Rusk & Cooper, 1994; Wise, Spindler, DeWit, & Gerber, 1978b). One study found evidence that the mixed D<sub>1</sub>/D<sub>2</sub> antagonists fluphenazine and pimozide, the D<sub>1</sub> antagonist SCH 23390, and the D<sub>2</sub> antagonist raclopride all significantly blocked the response to food reward (Fowler et al., 1994; Wise, Spindler, DeWit, & Gerber, 1978). These studies suggest that both the D<sub>1</sub> and D<sub>2</sub> receptors are involved in reward.

### Operant Conditioning Studies

Manipulations of the reward pathway by various agonists and/or antagonists has become a useful method for studying reward system functioning (Beninger et al., 1987; Wise, Spindler, DeWit, & Gerber, 1978). Because animals possess a natural desire to seek food, they readily learn to lever-press to obtain food pellets. Not surprisingly, the DA antagonist

pimozide, which has been used to suppress instrumental conditioned responding, also significantly attenuates operant conditioning for food (Beninger et al., 1987). Recent microdialysis studies confirm the role played by DA in the consummatory responses of food intake. Rats responding at a high rate for food reinforcement show a significant increase in extracellular DA and DA metabolites in the NA (Ettenberg & Camp, 1986; Salamone et al., 1994). Similarly, Kiyatkin and Gratton (1994) found increased NA DA levels shortly after cues were presented that signaled access to the food-lever. DA levels continued to increase as the subjects earned food reward. These DA levels remained high until lever pressing ceased, causing DA to return to basal levels (Kiyatkin & Gratton, 1994).

The operant conditioning paradigm has also been used to examine which DA receptor subtypes are involved in reward functioning. For example, operant conditioning for food reward is blocked by either D<sub>1</sub> and/or D<sub>2</sub> receptor antagonists, indicating that both D<sub>1</sub> and D<sub>2</sub> receptors are involved in reward (Beninger et al., 1987; Nakajima & Baker, 1989). Not surprisingly, injecting the selective D<sub>1</sub> DA antagonist SCH 23390 into the NA also results in the inhibition of lever-pressing for food (Beninger et al., 1987). Thus, DA antagonist studies show that the DA system is critically involved in reward and that both the D<sub>1</sub> and D<sub>2</sub> receptors are

necessary for normal reward functioning.

### DA Agonists and Operant Conditioning

Much of the work with psychomotor stimulant drugs, such as cocaine and amphetamine, have provided evidence that DA systems are involved in mediating the reinforcing properties of those drugs (Pettit, Ettenberg, Bloom, & Koob, 1981; Yokel & Wise, 1976). Rats continue to self-administer cocaine and amphetamine unless a DA antagonist is administered or the DA terminals in the NA are destroyed by toxic lesion (Yokel & Wise, 1976). Although amphetamine- and cocaine-induced reward correlates with an increase in extracellular DA in the NA, the mechanisms of these drugs appear to differ (DiChiara & Imperato, 1988; Ritz, Lamb, Goldberg, & Kuhar, 1987). Specifically, cocaine increases DA levels by blocking DA reuptake (DiChiara & North, 1992; Ritz et al., 1987; Svingos, Strecker, McNeish, & Hitzemann, 1991), whereas amphetamine increases DA release from the terminal fibers (DiChiara & Imperato, 1988).

Recently, D<sub>1</sub> receptors located in the NA have been directly implicated in the reinforcing effects of cocaine. Rats were given localized NA injections of a selective D<sub>1</sub> antagonist to evaluate DA involvement in the reinforcing properties of cocaine. The antagonism of this receptor caused significant increases in cocaine self-administration, which

suggests that D<sub>1</sub> receptors in the NA modulate cocaine self-administration (Maldonado, Robledo, Chover, & Caine, 1993). Curiously, Calcagnetti et al. (1995) have found that non-dopaminergic receptor antagonists can successfully attenuate cocaine's actions. They include calcium channel blockers, 5-HT<sub>3</sub> antagonists, and opiate antagonists. There is much speculation about the underlying mechanisms by which these drugs attenuate cocaine's actions. One possibility is that these drugs regulate ion flow through neuronal membrane channels (Calcagnetti et al., 1995; Menkens et al., 1992). For example, calcium blockade itself causes an attenuation in DA levels (Martellota, Kuzmin, Muglia, Gessa, & Fratto, 1994).

In summary, operant conditioning studies provide excellent evidence of DA's role in reward. DA antagonists significantly attenuate operant conditioning for reward (Beninger et al., 1987; Wise, Spindler, DeWit, & Gerber, 1978). Microdialysis and electrochemical examination of mesolimbic DA activity also supports DA's involvement in conditioned behaviors (Ettenberg & Camp, 1986; Kiyatkin & Gratton, 1994; Salamone et al., 1994). Although there is some disagreement as to the exact DA subtypes involved in reward, most agree that both D<sub>1</sub> and D<sub>2</sub> receptors are necessary for its expression (Beninger et al., 1987; Nakajima & Baker, 1989; Phillips et al., 1991).

## CPP

Along with instrumental conditioning and operant conditioning studies, CPP experiments implicate the mesolimbic DA system in drug-induced reward. Although instrumental conditioning and operant studies have provided considerable amounts of information on the neural substrates involved in reward, the disadvantage of these procedures is that animals must perform complex maneuvers while still under the influence of the drug (Ettenberg, 1989). The CPP paradigm eliminates this problem because conditioned animals are in a non-drugged state during testing (Bozarth, 1987; Schechter & Calcagnetti, 1993).

In the CPP paradigm rats are given a drug and confined to one side of a two compartment chamber (these compartments are visually and tactilly distinct). On the subsequent day the same rat is given saline and confined in the opposite chamber. After a number of trials, the animal is tested in the drug free state and their compartment preference is assessed. This paradigm eliminates the possible interference of motor effects caused by testing in the drug-induced state (Schechter & Calcagnetti, 1993). Evidence indicates that CPP can be established in rats by systemic injections of cocaine, amphetamine, and morphine. This reinforced behavior can be blocked by haloperidol and reduced by 6-



OHDA lesions (Hawkins & Stein, 1991; Reicher & Holman, 1977; Sherman, Roberts, Roskam, & Holman, 1980; Spyraiki, Fibiger, & Phillips, 1982a; Wise & Rompre, 1989). Studies have also shown that DA neurotransmission is essential for the establishment of the reinforcing properties of food reward, as well as the addictive qualities of substances of abuse (Beninger et al., 1987; Fibiger & Phillips, 1982; Wise, Spindler, & Legault, 1978; Wise & Bozarth, 1987).

Evidence indicates that reward is actually two separate processes that are controlled by, and function differently within the mesolimbic system (Hiroi & White, 1991a, 1991b; White & Carr, 1984, 1995). The acquisition and expression of reward behaviors are mediated by different receptor mechanisms (Hiroi & White, 1989, 1990, 1991a; Spyraiki, Fibiger, & Phillips, 1982a; White & Carr, 1984, 1985). The acquisition of a CPP involves the pairing of environmental stimuli with drug-induced mesolimbic DA activation. Whereas, the expression of a previously established CPP can be activated by conditioned stimuli, which are originally paired with events that had evoked the unconditioned release of DA. These two mechanisms (i.e., acquisition and expression) are dissociable. For example, acquisition and expression can be individually blocked by antagonists of different DA receptor subtypes (White & Carr, 1984, 1985). The acquisition of CPP appears to be dependent upon

activation of DA neurons within the VTA, resulting in DA release in the NA. Microinjections of DA agonists in this area results in CPP acquisition, and it is attenuated by the depletion of DA levels within the NA (DiChiara & Imperato, 1988; Spyraiki et al., 1982b). Studies of the expression of a previously established drug-induced CPP demonstrate that it can be blocked through the systemic, or NA injection of DA antagonists (Hiroi and White, 1989, 1990; Spyraiki et al., 1982b). When selective DA antagonists are injected following conditioning trials, the expression of CPP on the drug-free test day is effectively blocked (Hiroi & White, 1991a, 1991b).

More specifically, when conditioned stimuli (i.e., a scented or visually distinct chamber) are paired with an unconditioned stimulus (i.e., the DA release caused by the addictive drug), the conditioned stimuli eventually acquire the property of evoking DA neuronal activation. Thus, when the animal encounters conditioned stimuli in the absence of the drug, an elevation of DA metabolism is observed in the terminal area of the mesolimbic system (Hiroi & White, 1990, 1991a). This is thought to be the expression of the conditioned response. Evidence suggests that D<sub>1</sub> receptors located in the NA mediate the expression of CPP behavior (Hiroi & White, 1991a; White, Packard, & Hiroi, 1991). In contrast, evidence indicates that D<sub>2</sub> receptors in the NA

mediates the acquisition of the unconditioned effects of the addictive drug (Hiroi, McDonald, & White, 1990; Hiroi & White, 1989, 1990, 1991a, 1991b). This possibly explains why DA release is still observed when rats are given D<sub>2</sub> antagonists after cocaine and amphetamine treatment (Hiroi & White, 1991a; Ritz et al., 1987). The D<sub>1</sub> mediated conditioned reinforcers are not blunted by D<sub>2</sub> antagonists.

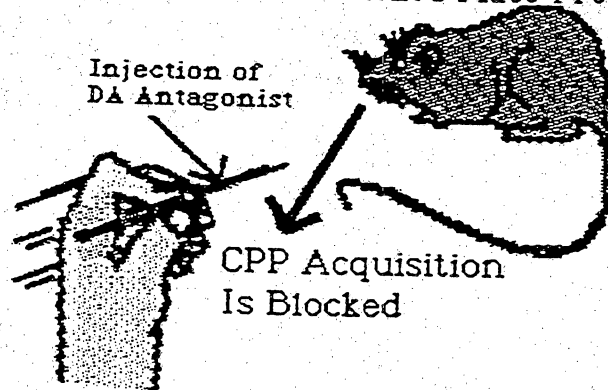
Further evidence suggests that the expression of conditioned behavior is also mediated through intrinsic neurons of the lateral nucleus of the amygdala, because NMDA toxic or electrolytic lesions made to this region attenuates amphetamine-induced CPP (Hiroi & White, 1991b). Afferents that target the NA project from the amygdala, hippocampus, entorhinal cortex, and the cerebral cortex (among others) (Kelley, Domesick, & Nauta, 1982; Phillipson & Griffiths, 1985; Sorensen & Witter, 1983) (see Figure 1 and 3). However, it is the amygdala and hippocampus that have been specifically implicated in memory, and lesions to these two regions cause deficits in several types of memory tasks (Peinado-Manzano, 1990; Sutherland & McDonald, 1990). In fact, Hiroi and White (1991) discovered that expression of amphetamine-induced CPP is mediated by intrinsic neurons of the lateral nucleus of the amygdala, since their destruction effectively prevents the expression of CPP reward (Hiroi & White, 1991b). This suggests that DA afferents

connecting the lateral nucleus of the amygdala and the NA, are responsible for the expression of CPP. Furthermore, 5-HT<sub>3</sub> receptors localized to the amygdala have recently been implicated in the modulation of reward (Dyr & Kostowski, 1995).

Although both the acquisition and expression of conditioned behaviors are mediated by DA receptor activation, different subtypes modulate these mechanisms (Hiroi & White, 1989, 1990; Spyraiki, Fibiger, & Phillips, 1982a; White & Carr, 1984, 1985). Studies have demonstrated that the drug-induced acquisition of CPP can be attenuated when D<sub>2</sub> antagonists are given prior to the DA agonist during conditioning sessions. This inhibits the acquisition of CPP, as demonstrated by the rat showing no preference behavior on the drug-free test day (Hiroi & White, 1989; Spyraiki, Fibiger, & Phillips, 1982a). Surprisingly, the acquisition of amphetamine-induced CPP can be initiated by the injection of selective agonists of either D<sub>1</sub> or D<sub>2</sub> receptors located within the NA. When antagonists of either subtype are injected before conditioning, the acquisition of CPP is blocked (Hoffman & Beninger, 1989; Leone & DiChiara, 1987; Spyraiki, Fibiger, & Phillips, 1982a) (see Figure 5). In contrast, the expression of previously established CPP can be inhibited by systemic or intracranial NA injections of D<sub>1</sub> antagonists just prior to the drug-free test trial.

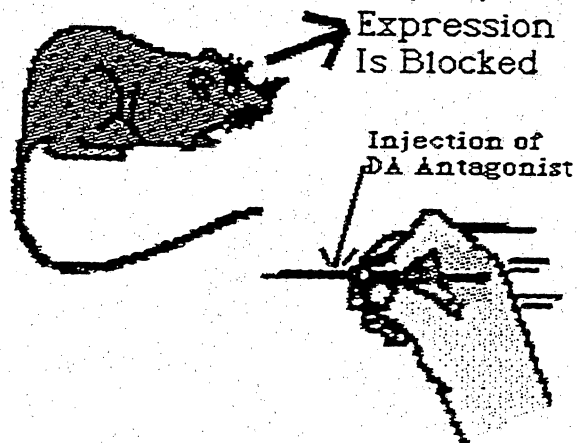
### BLOCKING ACQUISITION

DA Antagonist Is Given During Morphine- Or Cocaine-induced Conditioned Place Preference (CPP)



### BLOCKING EXPRESSION

DA Antagonist Is Given After Morphine- or Cocaine-induced Conditioned Place Preference (CPP)



### BLOCKADE OF THE ACQUISITION OR EXPRESSION OF CPP

Figure 5. Blockade Of CPP Acquisition Or Expression.

Furthermore, evidence indicates that antagonists of the D<sub>2</sub> receptor subtype are unable to block the expression of drug-induced CPP when injected following conditioning. Only antagonists of the D<sub>1</sub> receptor can prevent the expression of a previously established amphetamine-induced CPP (Hiroi & White, 1991).

The acquisition of CPP by cocaine has shown a similar complexity of action. Although cocaine-induced CPP can be blocked by the DA antagonist pimozide, it appears that the neural mechanisms mediating cocaine-induced CPP are complicated (deWit & Wise, 1978; Goeders & Smith, 1983). For example, some neuroleptics have no effect on the acquisition or expression of cocaine-induced CPP.

More specifically, haloperidol does not block cocaine-induced CPP except at very high doses, whereas low doses of haloperidol attenuate amphetamine-induced CPP (Hawkins & Stein, 1991; Spyraiki, Fibiger, & Phillips, 1982b; Wise & Rompre, 1989). Furthermore, lesions to DA neurons in the NA blocks cocaine self-administration, but not cocaine-induced CPP (Dworkin, Guerin, Goeders, & Smith, 1988; Spyraiki, Fibiger, & Phillips, 1982b).

In summary, CPP is an excellent paradigm for evaluating reward processes (Bozarth, 1987; Schecter & Calcagnetti, 1993). Drugs that increase DA levels, such as cocaine and amphetamine, induce the

acquisition and expression of CPP. Antagonists of either the D<sub>1</sub> or the D<sub>2</sub> receptor attenuate the acquisition of CPP, but only D<sub>1</sub> receptor antagonists attenuate the expression of CPP (Hawkins & Stein, 1991; Hiroi & White, 1991a; Leone & DiChiara, 1987; Reicher & Holman, 1977; Sherman et al., 1980; Spyraki, Fibiger, & Phillips, 1982a; Spyraki, Fibiger, & Phillips, 1982b; Wise & Rompre, 1989). Thus, two dissociable processes (i.e., acquisition and expression) are important for place preference conditioning; however, it appears that both of these processes are mediated by dissociable DA systems.

#### OPIOID REWARD

Operant conditioning and CPP studies also indicate the presence of a mesolimbic opioid system. Research indicates that a tonically active endogenous opioid system modulates the basal activity of mesolimbic DA neurons and, in this way, the opioid system has a marked effect on conditioned behaviors. Opiate-induced reward can be attenuated by DA receptor antagonists such as SCH 23390 and eticlopride, as well as the opioid antagonists naloxone and naltrindole (Bozarth, 1987; Bozarth & Wise, 1981; Hawkins & Stein, 1991; Phillips et al., 1991).

#### Operant Conditioning Studies

The operant conditioning paradigm has provided evidence that opioid receptors have a role in DA reward processes. The reinforced

responding induced by morphine self-administration increases DA within the NA (Shippenberg et al., 1993). Moreover, recent electrochemical techniques suggest that  $\mu$ -opioid activation within the VTA results in a dose-dependent increase in DA electrochemical signaling (Noel & Gratton, 1995).

The infusion of morphine directly into the VTA causes rats to self-administer increasing doses of the drug, as evidenced by a more rapid bar-pressing rate (Olds, 1992). This illustrates that the rewarding properties of morphine, as reflected in increased self-administration behavior, are due to activation of DA neurons in the VTA (Bozarth, 1987). Additionally, Rompre and Wise (1988) demonstrated specifically that A10 VTA DA neurons are activated by morphine. They implanted electrodes that allowed rats to self-administer electrical stimulation to A10 neurons. Systemically injected pimozide increased the threshold for self-stimulation, while morphine injected into the VTA reversed the effects of pimozide, causing animals to revert back to the previous pattern of self-stimulation. Naloxone blocked the reversal effects of morphine and the GABA agonist muscimol reinstated self-stimulation behavior, apparently because rats were motivated to increase morphine reinforcement (Rompre & Wise, 1988). This operant conditioning study again indicates that DA cells in the VTA are important for reward and



suggests that DA is involved in morphine-induced reward. The study also points to the role GABA receptors play in morphine-induced reward by demonstrating that a GABA agonist can block morphine's actions (Rompre & Wise, 1988).

### CPP

CPP studies also show that the endogenous opioid system is involved in reward. CPP can be induced by injecting D-ala-met-enkephalin (a specific  $\mu$  receptor agonist) directly into the VTA. Importantly, microdialysis has shown that this causes a concurrent increase in extracellular DA within the NA. Pretreatment with an opiate antagonist, such as naloxone or naltrindole, attenuates opiate-induced CPP, while at the same time suppressing DA levels in the NA (Barr & Rossi, 1992; Bozarth & Wise, 1980; DiChiara & North, 1992; Hawkins & Stein, 1991; Suzuki, Tsuji, Mori, & Misawa, 1995). This implicates the endogenous opioid system in reward and suggests that it is located within the VTA. *k* opioid subtypes are also involved in reward, since activation of *k* opioid receptors attenuates responding for morphine (Mucha & Herz, 1985; Suzuki et al., 1995). Specifically, pretreatment with the *k* agonist U-50,488H blocks the normal accumulation of morphine-induced DA in the NA, while completely abolishing morphine-induced CPP (Mucha & Herz, 1985). When taken together, these studies

indicate that stimulation of  $\mu$  receptors activates DA neurons, while  $k$  receptor stimulation inhibits DA neurotransmission.

In summary, examination of the neurochemical mechanisms of reward indicates that an endogenous DA reward system mediates the rewarding properties of food and water. The endogenous opioids are also involved in reward, apparently by activating the DA reward system. Instrumental conditioning, operant conditioning and CPP studies have shown that both an endogenous DA and opioid system are components of the reward pathway.

### SEROTONIN STRUCTURE AND FUNCTION

Surprisingly, evidence supports the concept that the reward system can be modified by experimental changes in serotonin neurotransmission (Bilsky & Reid, 1991; Carboni, Acquas, Frau, & DiChiara, 1989; Carboni, Acquas, Leone, Perezzi, & DiChiara, 1988; Higgins, Joharchi, Nguyen, & Sellars, 1992; Imperato & Angelluci, 1989). Therefore, an examination of serotonin neurobiology will be followed by studies assessing interactions between DA and serotonin systems within the reward process.

#### Serotonin Pathways

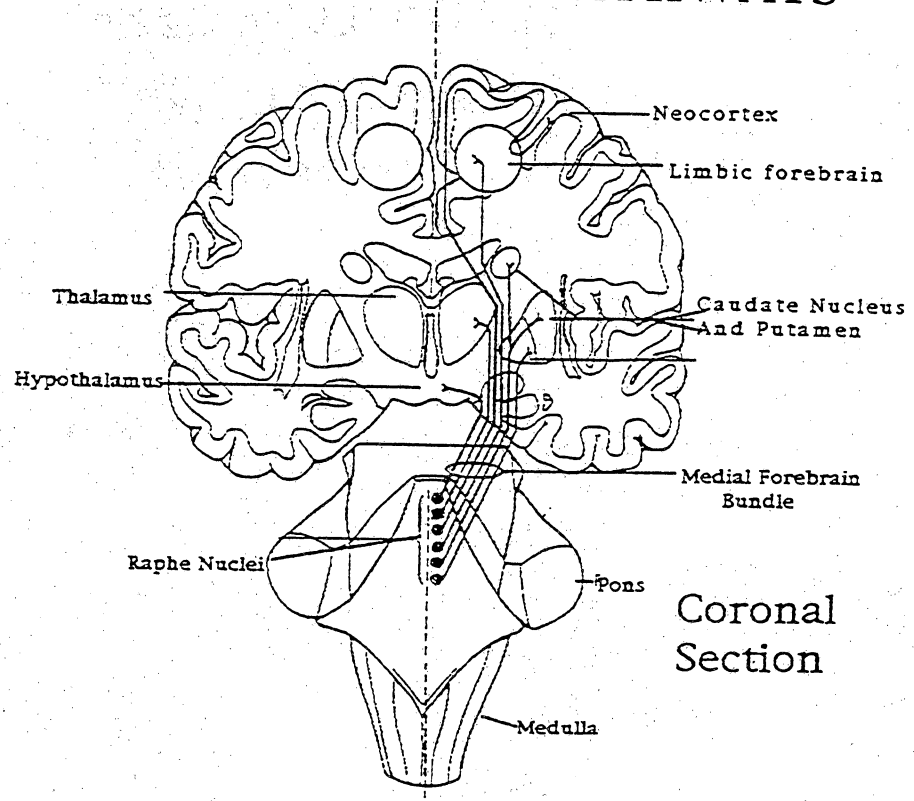
The connections made by serotonin neuronal processes throughout the brain indicates its potential to modulate DA neurotransmission. Serotonin axons innervate many of the same regions of the CNS where DA

pathways are found (Jacobs & Azmitia, 1992). Passing through the medial forebrain bundle, serotonin projections reach many rostral sites including the striatum, limbic structures, diencephalon and the cerebral cortex (Jacobs & Azmitia, 1992; Shizgal & Murray, 1989) (see Figure 6 and 7).

Although outnumbering noradrenergic and DA cells, serotonin cell bodies are localized to a few nuclei in the brain stem (Lauder & Bloom, 1975). Serotonergic neurons arise from distinct nuclei called the dorsal raphe and median raphe. Raphe nuclei lay directly along the midline of the brainstem from the medulla to the midbrain and their fibers have both ascending and descending pathways (Lauder & Bloom, 1975; Nicholls et al., 1992; Steinbusch, 1984). Projecting to the medulla, descending fibers are believed to modulate spinal sensory and motor neurons, and are involved in regulating motor movements as well as in the perception of pain (Parent, Descarries, & Beaudet, 1981). This apparently is the reason that application of a serotonin agonist causes excitability of motor pathways.

Studies of ascending fibers indicate that raphe nuclei in the pons and midbrain innervate the entire brain, projecting from the locus coeruleus to form part of the ascending reticular activating system.

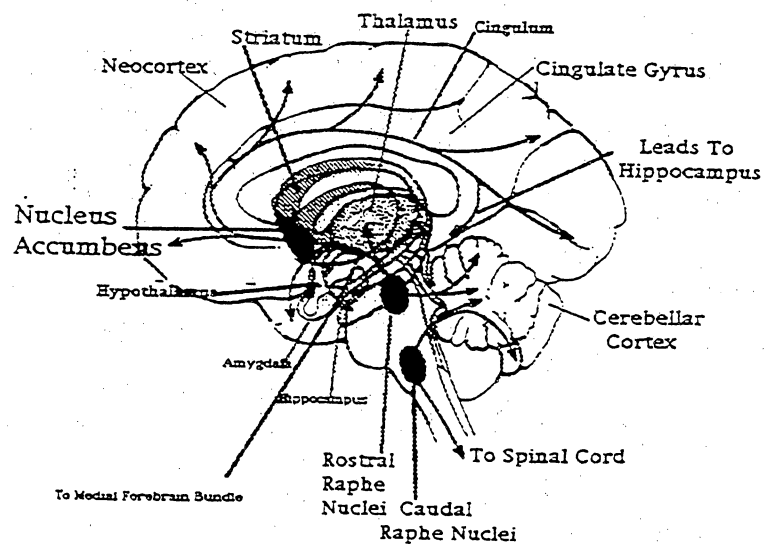
## SEROTONERGIC PATHWAYS



(Adapted from Kandel et al., 1995)

Figure 6. Serotonin Pathways: Coronal Section.

## Serotonergic Pathway Lateral View



(Adapted from Kandel et al., 1995)

Figure 7. Serotonin Pathways: Lateral View.

Thus, 5-HT neurons have been implicated in the sleep-wake cycle, through the modulation of locus coeruleus functioning and possibly other unknown mechanisms (Steinbusch, 1984).

The intraventricular administration of the serotonin tracer [3H]-5-hydroxytryptamine shows that ascending serotonin axons pass next to DA neurons (Parent et al., 1981). Similarly, serotonin-like cells and fibers are found in close proximity to A10 DA neurons in the VTA, indicating that mesolimbic DA neurons within the A10 area of the tegmentum appear to be innervated by serotonergic axons projecting from the pontine raphe (Halliday & Tork, 1989) (see Figure 8).

#### Serotonin Receptors: Distribution And Function

Researchers have attempted to detail the distribution of serotonin receptors through assays of genetic material, immunoreactive analysis and radiographic binding studies. Serotonin mRNA has been found in the NA, striatum, tegmentum, spinal cord, basal forebrain, nucleus basalis, olfactory tubercle and cerebral cortex (Johnson & Heinemann, 1995). Released from the terminal, serotonin neurotransmitter binds to its receptor binding site (see Figure 9).

## MESOLIMBIC DOPAMINE SYSTEM

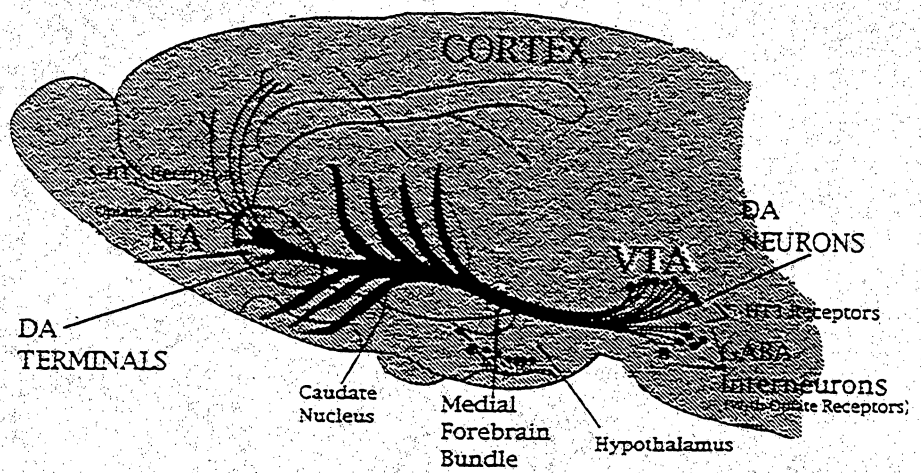
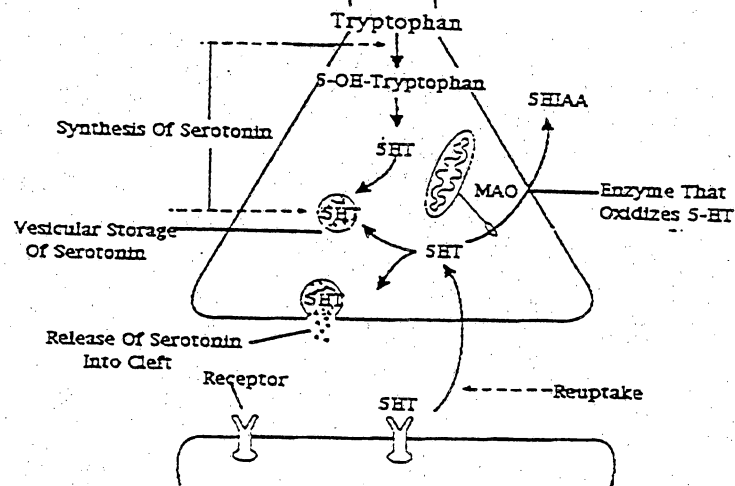


Figure 8. Mesolimbic Dopamine System Showing Various Receptor Locations.

## Serotonin Synthesis And Receptor Model



(Adapted from Kandel et al., 1994)

Figure 9. Serotonin Receptor Model.



Serotonin receptors are grouped into three basic families according to structure and second messenger system properties: 1) the 5-HT<sub>1</sub> family includes 5-HT<sub>1A</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub> and 5-HT<sub>1F</sub>, which utilize G-protein-mediated signal transduction; 2) the 5-HT<sub>2</sub> family includes 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub> and 5-HT<sub>4</sub>, which use phosphoinositol-mediated signal transduction; 3) the 5-HT<sub>3</sub> receptor which controls an ion-gated channel for signal transduction (Dubovsky & Thomas, 1995) (see Figure 10). Specifically, somatodendritic serotonergic autoreceptors of mainly the 5-HT<sub>1A</sub> type are found on serotonin neurons in the raphe nucleus and function to modulate firing of serotonergic neurons (Jacobs & Azmitia, 1992).

Autoreceptors located on terminals are of the 5-HT<sub>1D</sub> variety, and they modulate release of neurotransmitter from serotonergic terminals (Boess & Martin, 1994). Serotonin receptors located in the mesolimbic area include the 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> subtypes (Barnes, Barnes, Costall, Ironside, & Naylor, 1989; Boess & Martin, 1994; Jacobs & Azmitia, 1992).

Evidence implicates 5-HT<sub>3</sub> receptors in a presynaptic role, both in the peripheral nervous system (PNS) and the central nervous system (CNS). Besides being localized to presynaptic sites on fibers and terminals in the PNS, research suggests that the 5-HT<sub>3</sub> receptors are also located on axons at synaptic terminals in regions of the mesolimbic, nucleus tractus

solitarius, and medulla oblongata (Barnes, Barnes, Costall, Ironside, & Naylor, 1989; Kidd et al., 1993; Laporte et al., 1992). Importantly, autoradiographic studies have found that 5-HT<sub>3</sub> receptors appear to reside on fibers which project from serotonin cell bodies clustered in raphe nuclei. These fibers appear to pass near DA cells located in the midbrain (Barnes, Barnes, Costall, Ironside, & Naylor, 1989; Kidd et al., 1993) (see Figure 1). Biochemical, electrophysiological and behavioral studies indicate that 5-HT<sub>3</sub> receptors modulate the release of DA, serotonin, norepinephrine, acetylcholine and cholecystokinin (Barnes, Barnes, Costall, Naylor, & Tyers, 1989; Blandina, Goldfarb, Craddock-Royal, & Green, 1989; Grant, 1995).

The highest density of 5-HT<sub>3</sub> mRNA sequences and receptor binding is found in the nucleus tractus solitarius, dorsal motor nucleus of the vagus nerve, area postrema, spinal tract of the trigeminal nerve, and on all parts of the medulla oblongata (Cohen, 1990; Kilpatrick, Jones, & Tyers, 1988).

PCR amplification of mRNA 5-HT<sub>3</sub> sequences also indicates the presence of the 5-HT<sub>3</sub> receptor in the rat NA and VTA, and transcripts were detected in mouse embryo tegmentum as early as day 14 of gestation (Johnson & Heinemann, 1995; Maricq, Peterson, Brake, Myers, & Julius, 1991). Autoradiographic studies of the mesolimbic region appear

to confirm the presence of 5-HT<sub>3</sub> receptors in the NA and VTA. However, they are most dense in entorhinal cortex, hippocampus and amygdala, where it is believed that they exert their effects on cognition, anxiety, depression, psychotic symptoms, and the expression of reward (Boess & Martin, 1994; Hiroi & White, 1991b; Johnson & Heinemann, 1995; Maricq et al., 1991).

In summary, autoradiographic and mRNA evidence supports the close proximity of 5-HT<sub>3</sub> receptors to the DA reward pathway. Its presynaptic location and ligand-gating properties suggests a functional role in neurotransmitter release. Because this receptor is found both peripherally and in many regions of brain, systemic injection of 5-HT<sub>3</sub> agonists and antagonists have the potential of affecting not only the mesolimbic system but other systems as well.

#### DA AND SEROTONIN INTERACTIONS

Evidence supports the concept that serotonin receptors are important in modulating DA levels and release characteristics within the mesolimbic system (Carboni, Acquas, Frau, & DiChiara, 1989; Costall et al., 1976). When a serotonin agonist is given systemically, measurable increases of DA occur in the NA (Blandina et al., 1989; Blandina, Goldfarb, & Green, 1988; Jiang, Ashby, Kasser, & Wang, 1990; Yi, Gifford, & Johnson, 1991). In contrast, systemically injected 5-HT<sub>3</sub> antagonists

attenuate DA and serotonin agonist-induced increases of DA in the NA (Costall, Hui, & Naylor, 1979; Hagan et al., 1987; Imperato et al., 1992).

Conversely, DA levels appear to regulate serotonin release.

Serotonergic projections extending from serotonin cell bodies in the raphe nuclei, pass near dopaminergic cells clustered in the A10 region of the mesolimbic system. These serotonergic cells respond to changes in DA levels by adjusting the amounts of serotonin neurotransmitter they release (Barnes, Barnes, Costall, Ironside, & Naylor, 1989; Kandel et al., 1995; Kidd et al., 1993). Thus, the amount of DA in the VTA appears to be important in regulating serotonin production and release and, in turn, serotonin appears to regulate DA levels (Costall et al., 1987; Perry, Kostrzewa, & Fuller, 1995).

Experimentation on one of the earliest 5-HT<sub>3</sub> antagonists demonstrated that it produced inhibiting effects on DA agonist-induced reward behaviors in rats (Costall et al., 1987). Called ondansetron, this 5-HT<sub>3</sub> receptor antagonist inhibits amphetamine-induced locomotor activity. The attenuation of DA activation was achieved only when the 5-HT<sub>3</sub> antagonist was injected systemically or directly into the VTA and had no effect when localized injections were applied directly to the NA (Costall et al., 1979; Imperato & Angelucci, 1989). These studies caused heightened interest in the 5-HT<sub>3</sub> receptor and created a proliferation of

research on the role of 5-HT<sub>3</sub> receptors in modulating DA neurotransmission and reward (Acquas, Carboni, Grarau, & DiChiara, 1990; Bilsky & Reid 1991; Carboni, Acquas, Frau, & DiChiara, 1989; Carboni, Acquas, Leone, & DiChiara, 1989; Suzuki et al., 1992).

#### DA and Serotonin Interaction: Receptors

Evidence suggests that the serotonin receptor has a vital role in modulating DA functioning in the mesolimbic system. Serotonin agonists activate DA neurons in the VTA, resulting in increased DA within the NA, whereas antagonists attenuate these agonist-induced increases (Costall et al., 1987; Imperato & Angelucci, 1989; Invernizzi et al., 1995). Although serotonin antagonists attenuate the enhanced DA levels produced by DA and serotonin agonists, these antagonists have no effect on basal levels of DA (Costall et al., 1987; Invernizzi et al., 1995). This implies that serotonin functions in a “homeostasis-like” role to regulate DA levels in the mesolimbic system (Carboni, Acquas, Frau, & DiChiara, 1989; Costall et al., 1987; Imperato & Angelucci, 1989; Invernizzi et al., 1995; Pei, Zetterstrom, Leslie, & Grahame-Smith, 1993).

Cellular studies confirm the impact that 5-HT<sub>3</sub> agonists and antagonists have on the DA neuron. When applied to brain slices containing DA cells, the 5-HT<sub>3</sub> agonist 2-methylserotonin causes increased DA release that is blocked by the 5-HT<sub>3</sub> antagonist tropisetron

(Blandina et al., 1988, 1989). Although, 5-HT<sub>3</sub> agonists increase DA release, and 5-HT<sub>3</sub> antagonists block release, the mechanisms mediating these effects remain uncertain (Carboni, Acquas, Frau, & DiChiara, 1989; Costall et al., 1976; Gifford & Wang, 1994; Imperato & Angelucci, 1989). There are several possibilities: that 5-HT<sub>3</sub> drugs affect the DA transporter system, that 5-HT<sub>3</sub> receptors modulate DA neurons through receptors located near GABAergic interneurons, or that 5-HT<sub>3</sub> drugs directly bind to 5-HT<sub>3</sub> receptors located on DA terminals, which act to modulate DA release (Boess & Martin, 1994; Costall & Naylor, 1992; Klein et al., 1993; Yi et al., 1991).

First, evidence indicates that serotonin agonists may affect DA levels by binding to serotonin receptors, resulting in the modulation of the reuptake of DA into the presynaptic neuron. There is also an implication that 5-HT<sub>3</sub> receptors may block DA reuptake (Yi et al., 1991) (see Figure 11).

Although binding studies demonstrate the presence of 5-HT<sub>3</sub> receptors in the striatum and NA, direct injections of serotonin antagonists into these regions have no effect on DA content (Imperato & Angelucci, 1989; Yi et al., 1991). When a 5-HT<sub>3</sub> receptor agonist is applied to rat striatal cells, it induces DA release in synaptosomes, as well as a Ca<sup>++</sup>-evoked efflux.

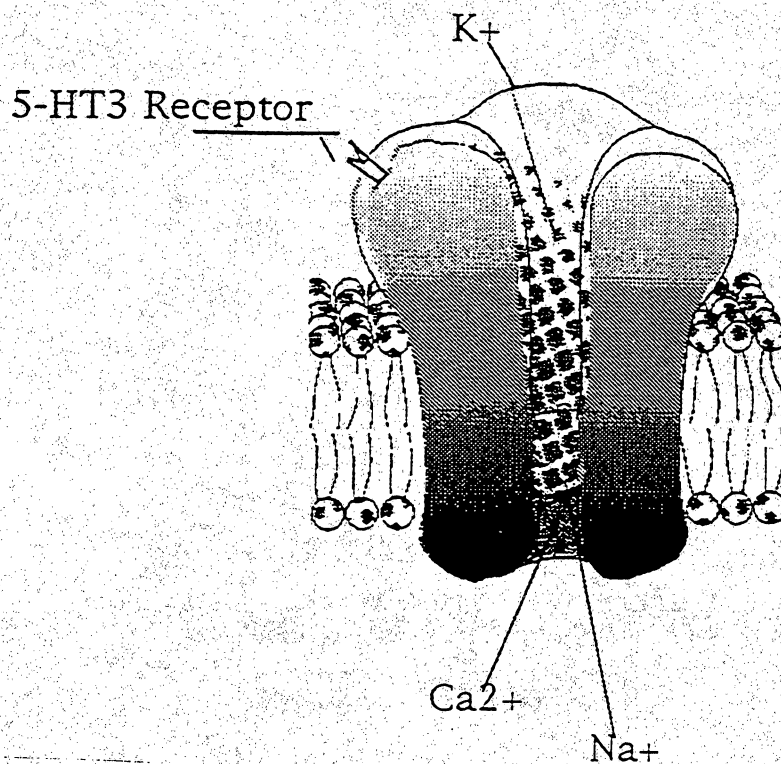


Figure 10. Serotonin 5-HT<sub>3</sub> Receptors Gate Ionic Channels.

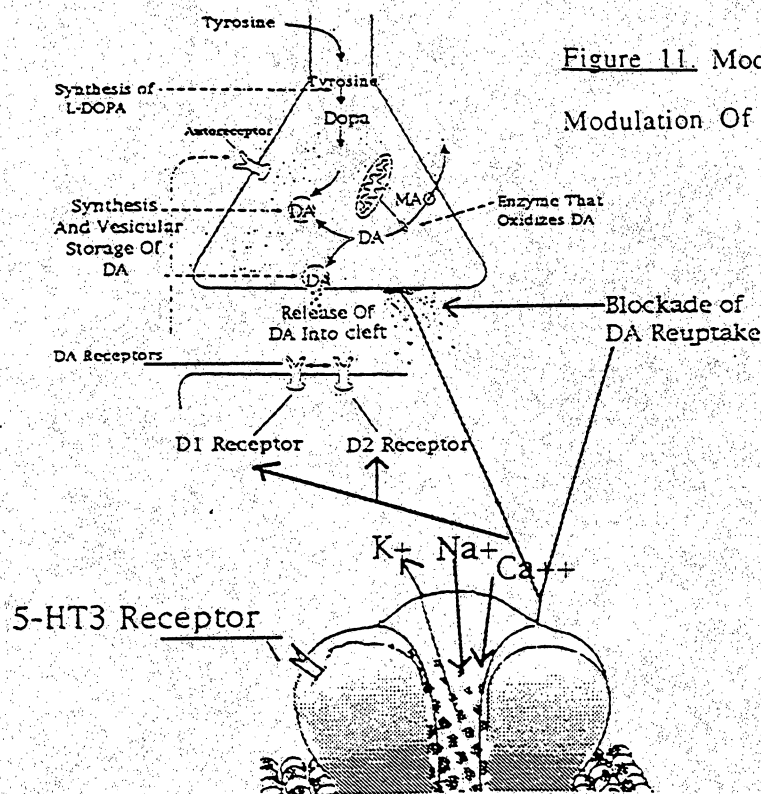


Figure 11. Model Of 5-HT<sub>3</sub> Modulation Of DA Reuptake.

Both of these effects can be antagonized by the selective DA uptake inhibitors nomifensine and cocaine. This implies that serotonin induces the release of DA from striatal cells by modulating DA reuptake into the DA terminal (Yi et al., 1991). Curiously, when a 5-HT<sub>3</sub> antagonist is applied to striatal cells, or is locally injected into striatum, there is no attenuation in the DA release produced by 5-HT<sub>3</sub> agonists (Costal et al., 1987; Yi et al., 1991). This suggests that action on the DA reuptake transporter may not be responsible for the actions of these serotonin drugs (see Figure 12).

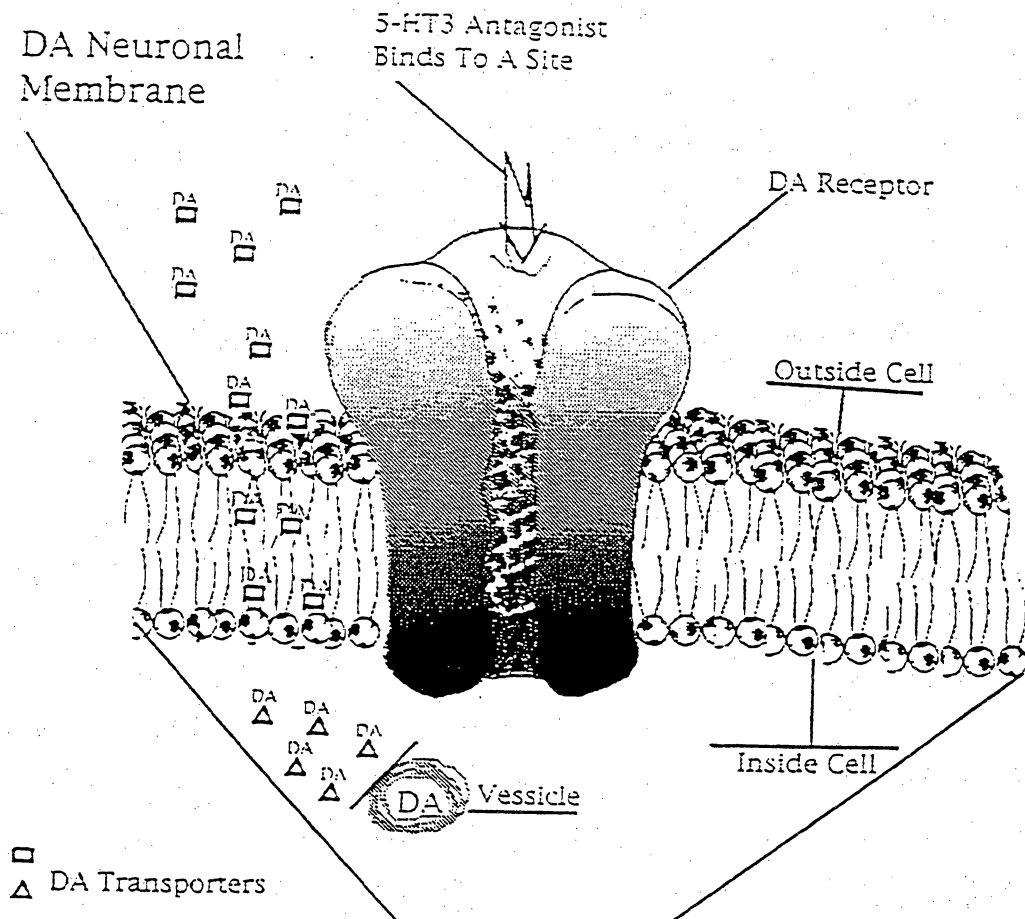
Second, the possibility remains that 5-HT<sub>3</sub> antagonists affect DA release by modulating GABA interneurons located in the VTA (Klein et al., 1993) (see Figure 13). For instance, Imperato and Angelucci (1989) demonstrated that the direct perfusion of 5-HT<sub>3</sub> antagonists into the NA had no effect on morphine's actions. To attenuate this drug-induced increase in DA, 5-HT<sub>3</sub> antagonists must be injected systemically or directly into the VTA where GABA interneurons can affect DA release (Imperato & Angelucci, 1989; Roth, 1994).

The third possibility is that activation of 5-HT<sub>3</sub> receptors, perhaps located on DA processes, may directly modulate DA release. mRNA and autoradiography studies suggest that 5-HT<sub>3</sub> receptors are present in both the NA and VTA.



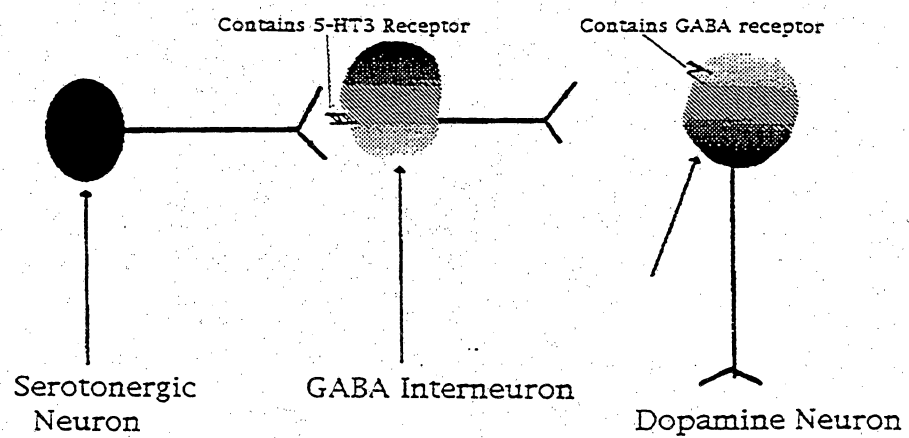
## Two Different Molecular Proteins May Transport DA

- Back Into The Presynaptic Terminal And
- Into The Vessicles



This Effectively Prevents The Vessicular Transporter From Transferring DA Back Into The Vessicle

Figure 12. Two Different Molecular Proteins That May Transport DA.



Model of Potential Interactions Between a GABA Interneuron and Serotonin and Dopamine Neurons

Figure 13. Model of Potential Interactions Between A GABA Interneuron and Serotonin and Dopamine Neurons.

Therefore, the possibility exists that 5-HT<sub>3</sub> agonists or antagonists are capable of directly affecting DA release by modulating presynaptic or postsynaptic sites on dopamine processes (Boess & Martin, 1994; Carboni, Acquas, Leone, & DiChiara, 1989; Leone & DiChiara, 1987; Roth, 1994; Yi et al., 1991).

If it is the 5-HT<sub>3</sub> receptor that is directly affected by 5-HT<sub>3</sub> drugs, then two questions still remain: 1) do these receptors reside on serotonin terminals projecting into the VTA or the NA, or 2) do other neurons or their processes contain these 5-HT<sub>3</sub> receptors. Serotonin antagonists and addictive drugs are being used to resolve these questions. They are capable of dissociating the mechanisms of the DA reward system. However, much remains to be learned. Although the rewarding effects of cocaine can be attenuated by 5-HT<sub>3</sub> antagonists, the receptor mechanisms responsible for this antagonism appear to be different than for other addictive drugs (Di Chiara & North, 1992). Microdialysis studies have suggested that while serotonin antagonists inhibit cocaine-induced CPP, DA levels remain unchanged in the NA (Svingos et al., 1991). Some believe that the serotonin antagonist's affinity for binding with 5-HT receptors may simply allow them to competitively displace cocaine. Successful attenuation by 5-HT<sub>3</sub> antagonism of cocaine-induced CPP can be accomplished when 10-fold doses are used, yet DA content remains

unchanged in the NA. This implies cocaine's actions may be mediated by other brain regions (Gifford & Wang, 1994; Paris & Cunningham, 1993; Peltier & Schenk, 1991).

Cellular studies have produced a different understanding of cocaine's action on the 5-HT receptor. Utilizing patch-clamp techniques on nodose ganglion cells, evidence demonstrates that the application of 5-HT (5-hydroxytryptamine) produces a fast inward current. When the 5-HT<sub>3</sub> antagonist MDL-72222 is pre-applied to the tissue, a blockade of this inward current is observed (Fan, Visentin, & Weight, 1994). The selective 5-HT<sub>3</sub> agonist 2-methyl-5-HT reinstates this inward current. When cocaine alone is applied, it has no effect on membrane current, but if cocaine and 5-HT are applied together, the serotonin-induced peak and steady-state current are reduced. If cocaine is pre-applied to the tissue before the 5-HT agonist, the peak and steady-state current is further depressed by cocaine. This suggests that cocaine inhibits 5-HT<sub>3</sub> receptor-mediated inward current (Fan et al., 1994; Lambert et al., 1989; Vanner & Surprenant, 1990). It also implies that some of cocaine's action may result from its competitive antagonism of 5-HT<sub>3</sub> receptors, resulting in a blockade of serotonin action.

In contrast to cocaine studies, experimental research has provided a clear understanding of the mechanisms in 5-HT<sub>3</sub> receptor antagonism

of morphine-induced reward. The prerequisite for the expression of morphine reward is the stimulation of DA release in the NA. Therefore, if 5-HT<sub>3</sub> antagonists can suppress drug-induced increases in DA, it supports a role for the serotonin receptor in the expression of the reward system (Imperato & Angelucci, 1989). Systemic injections of many 5-HT<sub>3</sub> antagonists, such as MDL-72222, ICS205-930 and ondansetron, cause a full or partial blockade of morphine-induced DA release in the NA (Carboni, Acquas, Leone, & DiChiara, 1989; Imperato & Angelucci, 1989; Pei et al., 1993). Similarly, direct injections into the VTA of ICS205-930, causes a reversal of the morphine-induced DA release in the NA (Imperato & Angelucci, 1989).

In summary, although evidence suggests that many 5-HT<sub>3</sub> agonists increase DA release in the NA (an action blocked by 5-HT<sub>3</sub> antagonists) the exact receptors mediating the DA/serotonin interactions remain uncertain (Carboni, Acquas, Frau, & DiChiara, 1989; Costall et al., 1976; Gifford & Wang, 1994; Imperato & Angelucci, 1989). Research has also shown that 5-HT<sub>3</sub> receptor antagonists are capable of attenuating morphine- and cocaine-induced reward (Carboni, Acquas, Leone, & DiChiara, 1989; Imperato & Angelucci, 1989; Pei et al., 1993).

#### DA and Serotonin Interaction: Rat Behavior

Behavioral studies utilizing injections of serotonin agonists and

antagonists have resulted in a greater understanding of the serotonin receptor's role in the DA reward pathway (Carboni, Acquas, Leone, & DiChiara, 1989; Carboni, Acquas, Frau, & DiChiara, 1989; Imperato & Angelucci, 1989; Suzuki et al., 1992). However, little is known about the involvement of serotonin receptors in the expression of reward. Most studies have tested only the acquisition of reinforced behavior (Acquas et al., 1990; Carboni et al., 1988; Higgins et al., 1992; Pei et al., 1993). Overall, acquisition of cocaine- and morphine-induced conditioned behaviors is blocked by antagonists of the 5-HT<sub>3</sub> receptor, while these antagonists fail to affect cocaine self-administration and stimulus discrimination (Borg & Taylor 1994; Calcagnetti et al., 1995; Carboni et al., 1988; Higgins et al., 1992; Joharchi et al., 1993; Martellota et al., 1994; Pei et al., 1993; Svingos & Hitzemann, 1992). For example, systemic injection of 5-HT<sub>3</sub> antagonists decrease cocaine-induced locomotion (Reith, 1990; Svingos & Hitzemann, 1992). Acquisition of cocaine-induced CPP was also attenuated by systemic treatment with 5-HT<sub>3</sub> antagonists (Suzuki et al., 1992; Svingos & Hitzemann, 1992). In contrast, 5-HT<sub>3</sub> antagonists only appear to block the locomotor activity produced by chronic, but not acute, cocaine treatment (King et al., 1994).

Along with blocking many cocaine-induced behaviors, 5-HT<sub>3</sub> antagonists attenuate morphine-induced responses in rats (Acquas et al.,

1990; Bilsky & Reid, 1991; Borg & Taylor 1994; Carboni et al., 1988; Higgins et al., 1992; Joharchi et al., 1993; Pei et al., 1993; Suzuki et al., 1992). 5-HT<sub>3</sub> antagonists not only inhibit certain conditioned behaviors, they attenuate morphine-induced DA increases in the NA. Consistent with this finding, 5-HT<sub>3</sub> antagonists attenuate morphine-induced CPP and locomotion (Carboni et al., 1988; Higgins et al., 1992; Pei et al., 1993). Interestingly, MDL-72222 and ondansetron were unable to block morphine self-administration (similar results were obtained with cocaine) (Borg & Taylor, 1994; Higgins et al., 1992). This suggests that regional specificity of drug effects may be involved in different behaviors (Svingos & Hitzemann, 1992).

In summary, studies examining the biochemical and behavioral actions of addictive drugs demonstrate that they produce reward by activating the mesolimbic DA system. The blockade of morphine- and cocaine-induced reward by antagonists of the 5-HT<sub>3</sub> receptor, clearly imply that this receptor is involved in the expression of reward. In addition, evidence has suggested that the biochemical consequences of 5-HT<sub>3</sub> antagonism is the inhibition of DA release in the NA. Thus, the antagonism of drug-induced reward behavior can be utilized to demonstrate the role of the 5-HT<sub>3</sub> receptor complex in mesolimbic reward.

## DEVELOPMENTAL IMPLICATIONS

### Ontogeny of Neurotransmitter Systems: DA

Although the behavioral responses may vary from those of the adult, it is apparent that D<sub>1</sub> and D<sub>2</sub> receptor activation has behavioral effects in preweanling rats. Receptor binding studies suggest that both D<sub>1</sub> and D<sub>2</sub> subtypes are present at birth and maintain a 1:1 ratio until 15 days postnatally (Gelbard, Teicher, Faedda, & Baldessarini, 1989; Giorgi et al., 1987; Sales, Martres, Bouthenet, & Schwartz, 1989). After this period, D<sub>1</sub> receptors begin to outnumber D<sub>2</sub> receptors, with an adult-like ratio occurring by about 60 days of age (Gelbard et al., 1989; Murrin & Zeng, 1986; Zeng, Hyttel, & Murrin, 1988). While the exact developmental implications of this disparity remains uncertain, some behavioral differences have been determined using psychopharmacological evidence.

Behavioral studies indicate that selective DA receptor agonists produce age-dependent behavioral differences in rats. For example, adult rats injected with the D<sub>1</sub> agonist SKF-38393 or the D<sub>2</sub> agonist quinpirole respond with increased grooming or vertical behavior, respectively (Moody & Spear, 1992). In contrast, rat pups given injections of SKF-38393 or quinpirole exhibit increased forward locomotion and/or sniffing behavior, while the typical adult behaviors are absent (Moody & Spear,



1992). Similarly, when rat pups under 17 days old are pretreated with the irreversible DA antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), the response to DA agonists differs from that of the adult rat. Unlike adults, rat pups fail to exhibit the attenuation of stereotyped behaviors induced by the nonselective agonist R-propylnorapomorphine (NPA) (McDougall & Bolanos, 1995; McDougall, Crawford, & Nonneman, 1992; Mestlin & McDougall, 1993). In addition, preweanling rats, when compared to adults, show a greater behavioral response to psychomotor stimulants like cocaine (Spear & Brick, 1979).

In spite of these ontogenetic differences, evidence suggests the presence of functional D<sub>1</sub> and D<sub>2</sub> receptors in preweanling rats (Moody & Spear, 1992). Nonetheless, it is important to understand the ontogenetic behavioral differences that can be elicited by different combinations of D<sub>1</sub>, D<sub>2</sub> or D<sub>1</sub>/D<sub>2</sub> receptor agonist stimulation.

#### Ontogeny of Neurotransmitter Systems: Opiates

Evidence indicates that rats as young as 5 days old have functional opioid receptors and possess the ability to respond behaviorally to opiate drugs (Kehoe & Blass, 1986). Binding studies demonstrate that opiate receptors are present and functional 15 days following birth (Bayon, Shoemaker, Bloom, Mauss, & Guillemin, 1979; Khachaturian, Alessi, Munfakh, & Watson, 1983).

Pharmacological responding can also be an indicator of neonatal opiate receptor maturity. Potential maturity can be assessed by measuring the ability of rat pups to exhibit morphine-induced CPP (Barr & Rossi, 1992). When a morphine dose, corresponding to the lowest effective adult dose, is injected directly into the VTA of 4-day-old rats, CPP is established. As in adult rats, only localized injections into the VTA, and not the NA or other brain areas, produces CPP in rat pups (Barr & Rossi, 1992). This implies that the neonatal reward system is functional shortly after birth and that opiate reward depends upon VTA activation (Laviola et al., 1992; Moran et al., 1981; Phillips & LePiane, 1980).

Although adult and preweanling rats exhibit morphine-induced reward, evidence suggests that drug-induced motor behaviors vary considerably (Caza & Spear, 1980). Specifically, a high (5.0 mg/kg) dose of morphine causes a decrease in locomotion and a low (1.0 mg/kg) dose increases locomotor activity in preweanling rats. In contrast, rats older than 17 days exhibit a reversal of this behavioral pattern (Caza & Spear, 1980; Spear & Brake, 1983). Importantly, morphine produces CPP in rats as young as 4 days of age (Barr & Rossi, 1992).

Importantly, CPP, as evidenced by time spent in the morphine conditioned environment, can be induced through localized VTA or

systemic injections shortly after birth (Barr & Rossi, 1992). Evidence indicates that rats have functional opiate receptors early in the preweanling period and are capable of performing morphine-induced CPP in a manner similar to adults (Barr & Rossi, 1992; Bayon, Shoemaker, Bloom, Mauss, & Guillemin, 1979; Khachaturian et al., 1983; Laviola et al., 1992; Pruitt et al., 1995).

#### Ontogeny of Neurotransmitter Systems: Serotonin

There is little research assessing the ability of serotonin antagonists to block DA agonist-induced behavior or neurochemical effects in neonatal rats (Pranzatelli, 1992). However, available evidence supports the concept that serotonin is present early in the prenatal period and that some receptor subtypes are functional at birth (Pranzatelli, 1992; Pranzatelli, Durkin, & Barkai, 1994). Research suggests that one of the first neurochemical systems to differentiate during ontogeny is serotonin. In the rat, serotonin neurons develop by day 13 of gestation and the serotonin neurotransmitter system becomes functional near the end of term (Eaton, Staley, Mordecai, Globus, & Whittemore, 1995; Whitaker-Azmitia, 1991). Research also suggests that serotonin is synthesized by raphe fetal cells and performs specific functions early in gestation. Serotonin may actually be produced before synaptic processes are needed, as evidenced by the fact that the fetus has serotonergic neurons

containing enzymatic material well before there is synaptic contact made at the terminal level. This suggests that serotonin may actually be synthesized and released within cells of the CNS to function in developmental processes (Eaton et al., 1995; Whitaker-Azmitia, 1991). For example, serotonin may guide the development of its own neurons (Eaton et al., 1995) and instigate and control neurite growth (Whitaker-Azmitia, 1991).

Receptor binding studies demonstrate the presence of serotonin receptors in rat pups and pharmacological research suggests functional responsiveness shortly after birth (Daval et al., 1987; Hard & Engel, 1988; Johansson-Wallsten et al., 1993; Pranzatelli et al., 1992, 1994). Utilizing tracer and radiographic techniques, it was found that the 5-HT<sub>1A</sub>, 5-HT<sub>2</sub> and 5-HT<sub>1C</sub> serotonin subtypes are present at birth (Daval et al., 1987). Serotonin receptor systems are capable of mediating behavior in neonatal rats. For example, rat pups have an increased sensitivity to the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT, as indicated by rapid involuntary muscle jerks and an increase in myoclonus. This is what one would expect from a compensatory serotonin (5-HT<sub>1A</sub>) supersensitivity or increased receptor density from repeated exposure (Ashby, Zhang, Edwards, & Wang, 1994; Hard & Engel, 1988; Pranzatelli et al., 1992, 1994). However, studies directly measuring preweanling rat sensitivity

and responsiveness to 5-HT<sub>3</sub> agonists or antagonists are nonexistent (Boess & Martin, 1994; Johansson-Wallsten, Berg, & Meyerson, 1993). Nonetheless, evidence suggests that 5-HT<sub>3</sub> receptors are present shortly after birth, and that the ability of neonates to respond behaviorally to other serotonin agonists is also present a few days following birth (Johansson-Wallsten et al., 1993). For example, serotonin agonists DOI, 8-OH-DPAT and fenfluramine are capable of inducing psychopharmacological responsiveness from birth to periadolescence, and no discontinuities in behavior have been observed (Hard & Engel, 1988; Johansson-Wallsten et al., 1993; Spear & Brake, 1983).

In summary, research suggests that preweanling rats possess functional DA, opiate and serotonin receptors, providing them with the ability to develop morphine- and cocaine-induced CPP. It is uncertain, however, whether serotonin receptor antagonism is capable of disrupting morphine- and cocaine-induced CPP in preweanling rats. Such a disruption occurs in adult rats, but the effects of serotonin antagonism on reward have not yet been assessed during the preweanling period.

#### GENERAL PURPOSE AND HYPOTHESIS

Many drugs of abuse share the common ability to increase extracellular DA levels in the mesolimbic system. It has been suggested that this may actually be the source of their rewarding properties

(DiChiara & Imperato, 1988). More specifically, the neuronal consequences of cocaine and morphine involve their ability to increase extracellular DA levels within the mesolimbic DA terminal regions, presumably due to the excitation of A10 DA neurons in the VTA (Gysling & Wang, 1983; Leone & DiChiara, 1987). The behavioral consequences of cocaine and morphine involve their ability to induce a state of reward which can be assessed via the CPP paradigm (Bozarth, 1987; Carboni, Acquas, Leone, & DiChiara, 1989; Higgins et al., 1992). In adult rats, acquisition of cocaine- and morphine-induced CPP can be blocked by prior treatment with the 5-HT<sub>3</sub> antagonist MDL-72222. To date, it is unknown whether MDL-72222 will block the expression of cocaine and morphine CPP. MDL-72222 appears to exert its inhibitory effects on reward functioning by its ability to ultimately decrease DA release in the NA (Acquas et al., 1990; Bilsky & Reid, 1991; Carboni, Acquas, Frau, & DiChiara, 1989; Carboni, Acquas, Leone, & DiChiara, 1989; Suzuki et al., 1992). Unfortunately, it is not known in which brain area these critical 5-HT<sub>3</sub> receptors are located. Moreover, it has not yet been determined whether 5-HT<sub>3</sub> antagonists are capable of modulating the acquisition and expression of cocaine- and morphine-induced reward in the preweanling rat.

Therefore, the purpose of this study is two-fold: first, to determine

whether systemically injected MDL-72222 (a 5-HT<sub>3</sub> antagonist) will block the expression of a previously established cocaine- or morphine-induced CPP in preweanling rats. Second, if MDL-72222 does not block the expression of CPP then we will determine whether systemically injected MDL-72222 is capable of attenuating the acquisition of cocaine- or morphine-induced CPP in preweanling rats.

The exact hypotheses are as follows. (1) Both morphine and cocaine are hypothesized to produce CPP in preweanling rats. (2) Systemic treatment with a full dose range of MDL-72222 is predicted to block the expression of morphine- and cocaine-induced CPP. If systemically injected MDL-72222 does not affect the expression of CPP (see hypothesis 2) then I will assess whether MDL-72222 blocks the acquisition of CPP. (4) It is hypothesized that a full dose range of MDL-72222 will attenuate the acquisition of morphine- and cocaine-induced CPP.

## EXPERIMENT 1

### Method

Subjects. Subjects were 122 (n=7-11) male and female rats of Sprague-Dawley descent (Harlan Industries, Indianapolis, IN, USA). The rats were bred and raised at California State University, San Bernardino. Litters were culled to a maximum of 10 pups at 3 days of age. The colony room was maintained at 20-23° C and kept under a 12:12 h light:dark cycle. Rats were tested when 17-19 days old.

Apparatus. The testing apparatus was a rectangular plywood chamber that has three compartments separated by removable partitions. The two end compartments measure 15 X 15 X 21 cm high, while the middle compartment measures 9 X 15 X 21 cm high. All compartments are painted gray and are covered by a clear Plexiglas top. One end compartment has rubberized non-slip flooring, whereas the other end compartment has plywood flooring scored (1 cm deep) in a checkerboard fashion. The middle compartment has smooth plywood flooring. Besides the tactile differences, both end compartments are equipped with an odor delivery system. More specifically, beneath each end compartment, and connected via 15 small holes in the floor, are rectangular plastic containers (14 X 7 X 4 cm high) partially filled with pinewood chip bedding. Lemon and almond extracts are applied to the



pinewood bedding of each container to provide distinctive odor cues for the end compartments (10 cc of the extract is used for conditioning and 1 cc is used for preference testing). During conditioning, solid partitions are used to keep rats in the appropriate compartments, whereas during testing the partitions are raised 5.5 cm so that each rat can move freely between the compartments.

Procedure and Drugs. Rat pups were given two 30 min conditioning trials on each of the two consecutive days. On the first conditioning day, the rats were given one saline injection and placed in a novel scented compartment for 30 min. This was repeated 4 hours later. On the second day, the rat pups were injected i.p. with saline, cocaine (20 mg/kg), or morphine (0.5 mg/kg), placed in a chamber for 30 min, and presented with a novel odor. The identical procedure was repeated four hours later. Importantly, drug treatment and chamber scent (lemon or almond) were counter-balanced so that scent alone would not confound results.

Following the two days of CPP conditioning, rat pups were injected with saline or the selective serotonin 5-HT<sub>3</sub> receptor antagonist MDL-72222. Thirty minutes later rats were given free access to both scented chambers for 15 min. It was predicted that MDL-72222 would block the expression of the cocaine- and morphine-induced place preference (see

Figure 14 for a summary of this experiment).

Data Analysis. Both conditioning sessions and testing sessions were video taped for behavioral assessment by a rater blind to treatment conditions. Total time spent in each compartment was measured in each treatment and control condition. Total time spent in the drug-paired compartment and locomotor activity (line-crosses) was analyzed using t-tests and analyses of variance (ANOVAs). Significant main effects and interactions were analyzed using Tukey tests.

Research Setting. Behavioral assessment was done in the vivarium.

## Results

Locomotor Activity. The mean locomotor activity counts of morphine-, cocaine-, and saline-treated rats during conditioning are shown in Figure 15. Overall, cocaine-treated rats had significantly more line-crosses than saline- or morphine-treated rats,  $F(2,116) = 80.54$ ,  $P < .05$ .

Conditioned Place Preference. MDL-72222 (0.17, 0.5, 1.5, 4.5 mg/kg, IP) failed to significantly block the expression of either morphine- or cocaine-induced CPP in 17-day-old rats (see Figures 16 and 17). More specifically, test day treatment with MDL-72222 did not affect the compartment preference of morphine- or cocaine-treated rats,  $F(4,40) = 1.40$ ,  $P > .05$ ;  $F(4,37) = .405$ ,  $P > .05$ ; respectively.

# EXPRESSION EXPERIMENT

## Systemic Injections

(MDL72222 Given Post-conditioning)

Drugs	MDL72222 0.0 mg/kg i.p.	MDL72222 0.17 mg/kg i.p.	MDL72222 1.5 mg/kg i.p.	MDL72222 0.5 mg/kg i.p.	MDL72222 4.5 mg/kg i.p.
Coc 20.0 mg/kg i.p.					
Morph 0.5 mg/kg i.p.					
Saline i.p.					

Figure 14. Protocol Design of Expression Experiment.

## LOCOMOTOR ACTIVITY

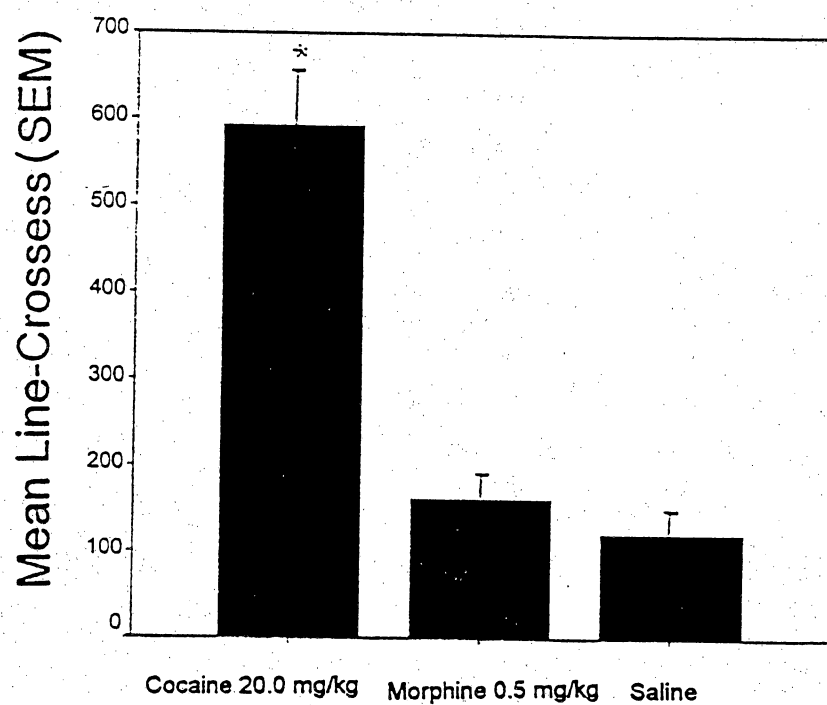


Figure 15. Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each). The 17-day-old rat pups were injected with cocaine (20.0 mg/kg, IP), morphine (0.5 mg/kg, IP), or saline immediately prior to conditioning (n=7-11).

# EXPRESSION OF MORPHINE CPP FOLLOWING DOSES OF MDL-72222

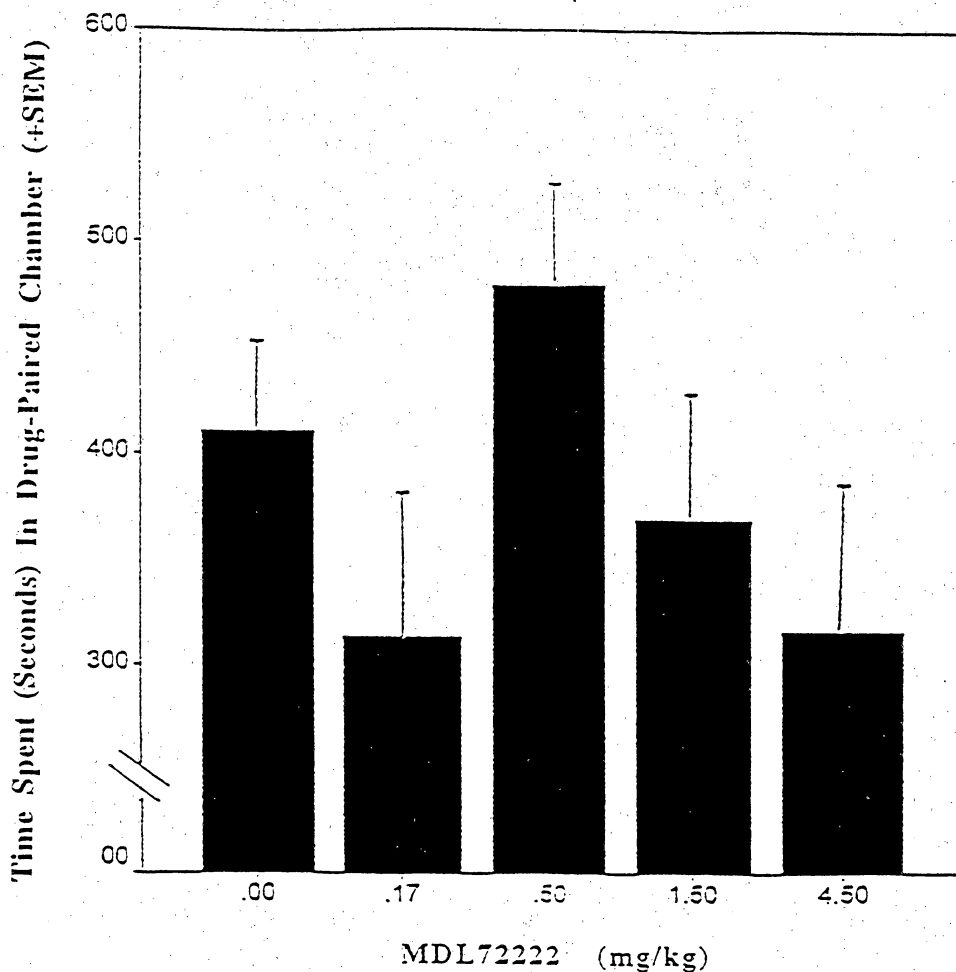


Figure 16. Mean time that rats spent (seconds) in the morphine-paired chamber (+SEM) of the CPP apparatus on test day. Thirty min following MDL-72222 (0.0, 0.17, 0.5, 1.5, 4.5 mg/kg, IP) treatment each 17-day-old rat pup was allowed 900 sec access to the three compartments (n=6-10).

EXPRESSION OF COCAINE CPP FOLLOWING DOSES OF  
MDL72222

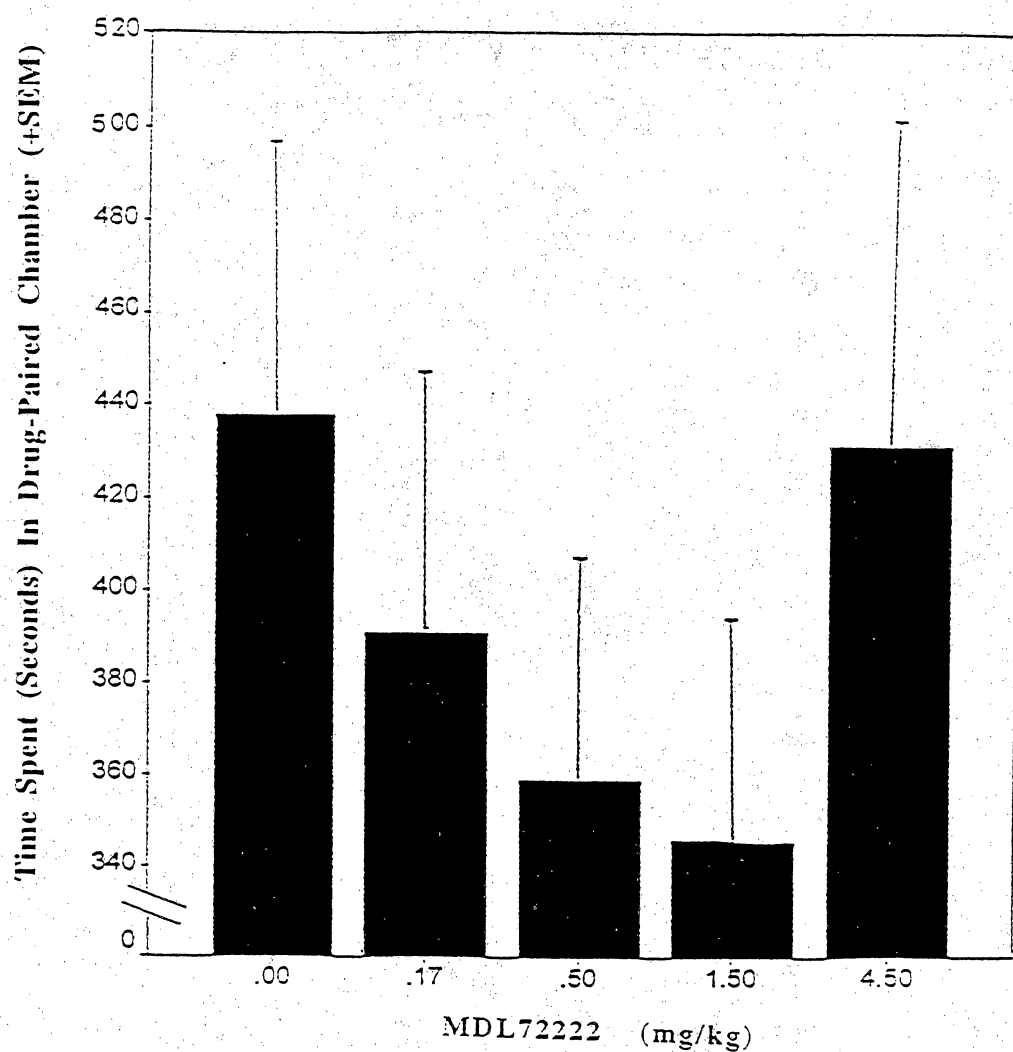


Figure 17. Mean time that rats spent (seconds) in the cocaine-paired chamber (+SEM) of the CPP apparatus on test day. Thirty min following MDL-72222 (0.0, 0.17, 0.5, 1.5, 4.5 mg/kg, IP) treatment each 17-day-old rat pup was allowed 900 sec access to the three compartment (7-10).

Surprisingly, test day treatment with MDL did affect compartment preference in saline-conditioned rats (see Figure 18). Post hoc comparisons using Tukey tests ( $P < .05$ ), showed that rats receiving the 4.5 mg/kg MDL-72222 exhibited a compartment preference.

Importantly, morphine and cocaine did produce a place preference (see Figures 19 and 20). Rats treated with 0.5 mg/kg of morphine spent significantly more total time in the drug-paired chamber when compared to saline treated rats,  $t(15) = -3.35$ ,  $P < .05$ . Similarly, the cocaine group spent significantly more total time in the drug-paired compartment than did rats receiving saline alone,  $t(14) = -2.77$ ,  $P < .05$ .

### Summary

MDL-72222, at dosage levels of 0.17 to 4.5 mg/kg, did not significantly affect the expression of morphine- or cocaine-induced CPP. Whereas, morphine or cocaine (given without MDL-72222) was able to induce CPP. Surprisingly, MDL-72222 (at the highest dose) did produce a compartment preference.

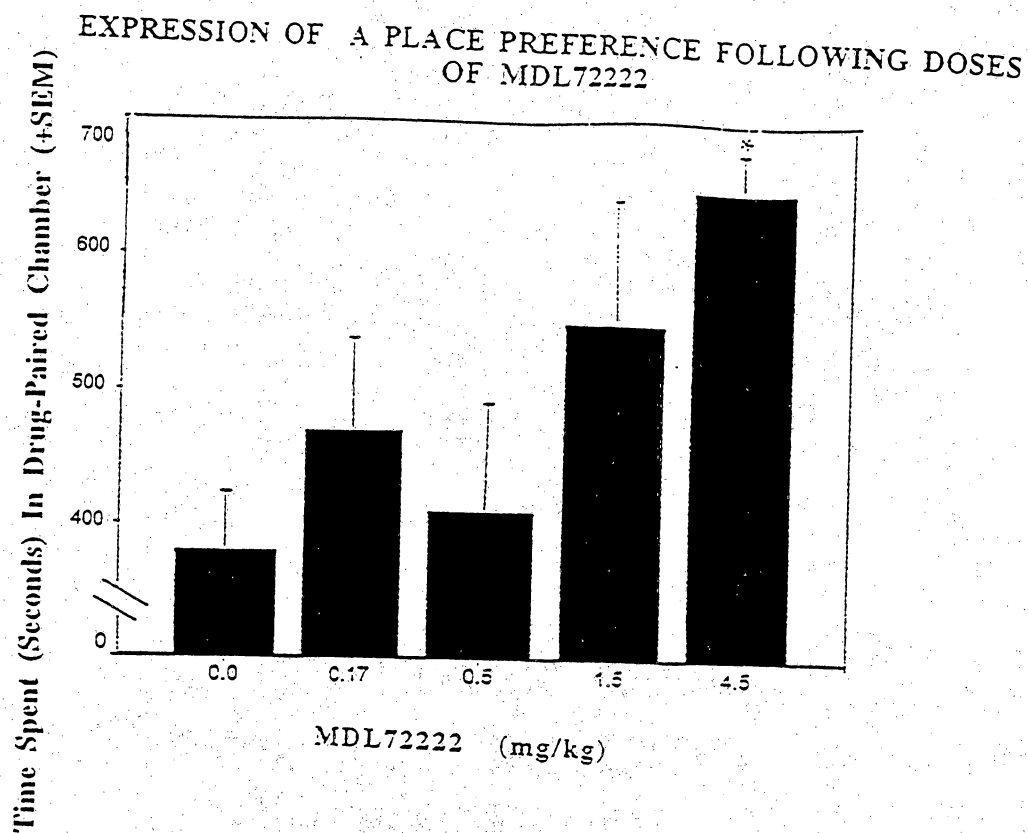


Figure 18. Mean time spent (seconds) in the saline-paired chamber (+SEM) of the CPP apparatus on test day, 30 min following a dose of MDL72222 (0.17, 0.5, 1.5, 4.5 mg/kg, IP) each 17-day-old rat pup was allowed 900 sec access to the three compartments (n=6-8).



## EXPRESSION OF MORPHINE CPP

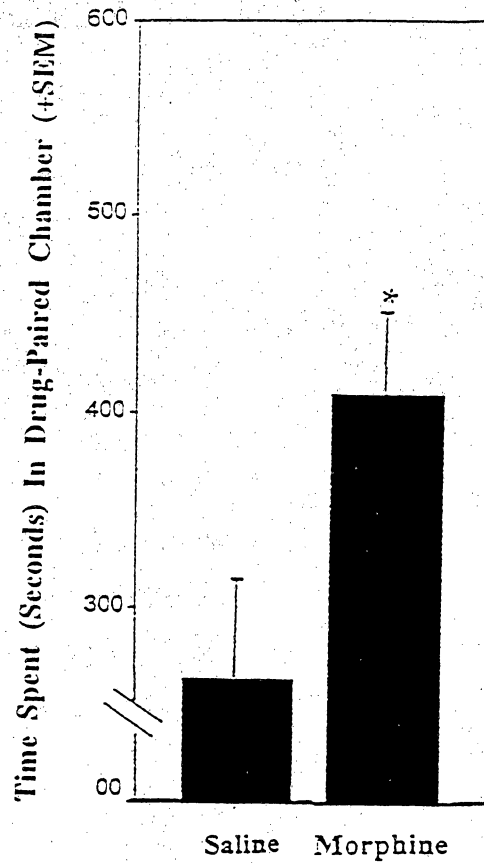


Figure 19. Mean time spent (seconds) in the preferred chamber (+SEM) by saline- or morphine-treated rat pups on test day. The 17-day-old rats were placed into the CPP apparatus 30 min following saline injections and allowed 900 sec access to all three compartments (n=7-11).

## EXPRESSION OF COCAINE CPP

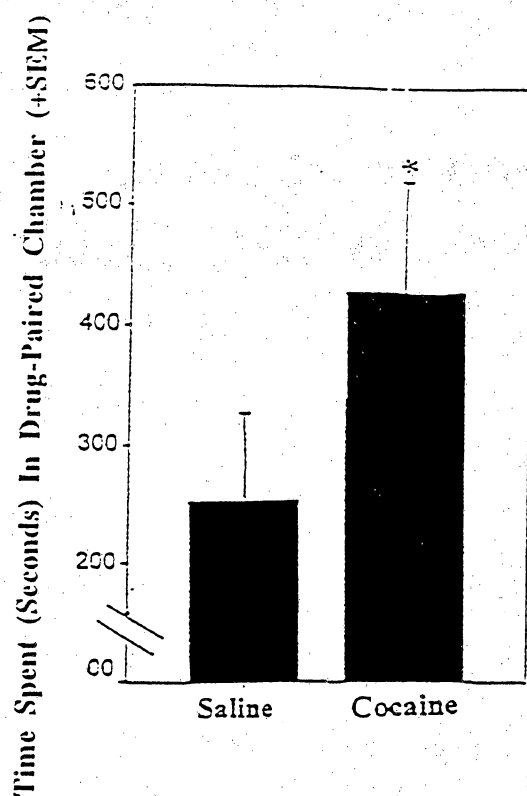


Figure 20. Mean time spent (seconds) in the preferred chamber (+SEM) by saline- or cocaine-treated rat pups on test day. The 17-day-old rats were placed into the CPP apparatus 30 min following saline injections and allowed 900 sec access to all three compartments (n=7-9).

## EXPERIMENT 2

### Method

Subjects. Total subjects numbered 84 (n=7). Otherwise the subjects and apparatus were the same as described in Experiment 1.

Procedure and Drugs. Rat pups were given two 30 min conditioning trials on each of the two consecutive days. On the first conditioning day, the rats were given two saline injections and placed in a novel scented compartment for 30 min. This was repeated 4 hours later. On the second day, 17-day-old rat pups were injected i.p. with saline or the selective serotonin 5-HT<sub>3</sub> receptor antagonist MDL-72222 (0.5, 1.5, 4.5 mg/kg). Thirty minutes later rats were given a second injection (i.p.) of saline, cocaine (20 mg/kg) or morphine (0.5 mg/kg), placed in a chamber for 30 min, and presented with a novel odor. The identical procedure was repeated four hours later. Importantly, drug treatment and chamber scent (lemon or almond) were counter-balanced so that scent alone would not confound results (see Figure 21) for a summary of this experiment)

# ACQUISITION Experiment

## Systemic Injections

(MDL72222 Given Pre-conditioning)

Drugs	MDL72222 0.0 mg/kg i.p.	MDL72222 0.5 mg/kg i.p.	MDL72222 1.5 mg/kg i.p.	MDL72222 4.5 mg/kg i.p.
Coc 20.0 mg/kg i.p.				
Morph 0.5 mg/kg i.p.				
Saline i.p.				

**Figure 21.** Protocol Design of Acquisition Experiment.

Following the two days of CPP conditioning, rat pups were injected with saline and were given free access to both scented chambers for 15 min. Pups conditioned with cocaine-alone or morphine-alone should prefer the drug-paired compartment. It was predicted that MDL-72222 would block the acquisition of this cocaine- and morphine-induced preference.

## Results

Locomotor Activity. The mean locomotor activity counts of morphine-, cocaine-, and saline-treated rats are shown in Figure 22, 23 and 24). Each rat pup had received a dose of MDL-72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) 30 min prior to the drug injections. Overall, cocaine-treated rats had significantly more line-crosses than saline- or morphine-treated rats,  $F(2,72) = 90.19$ ,  $P < .05$  (see Figure 23). MDL-72222 did not affect the locomotor activity of the morphine-, cocaine-, or saline-treated rats.

Conditioned Place Preference. MDL-72222 (0.17, 0.5, 1.5, 4.5 mg/kg, IP) failed to significantly block the acquisition of either morphine- or cocaine-induced CPP in 17-day-old rats (see Figures 25 and 26). More specifically, MDL-72222 given prior to injections of morphine (0.5 mg/kg, IP) or cocaine (20.0 mg/kg, IP) during conditioning did not affect compartment preference on the test day,  $F(3,24) = .02$ ,  $P > .05$ ;  $F(3,24) = .35$ ,  $P > .05$ ; respectively.

## LOCOMOTOR ACTIVITY

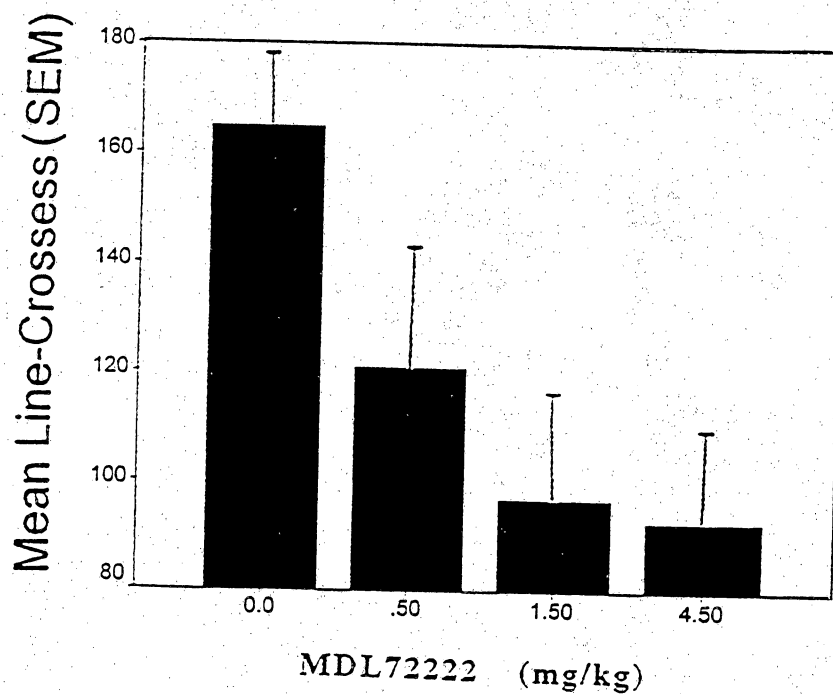


Figure 22. Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each). The 17-day-old rat pups were injected with morphine (0.5 mg/kg, IP), or saline 30 min following a dose of MDL72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) and placed into the conditioning chamber (n=7).

## LOCOMOTOR ACTIVITY

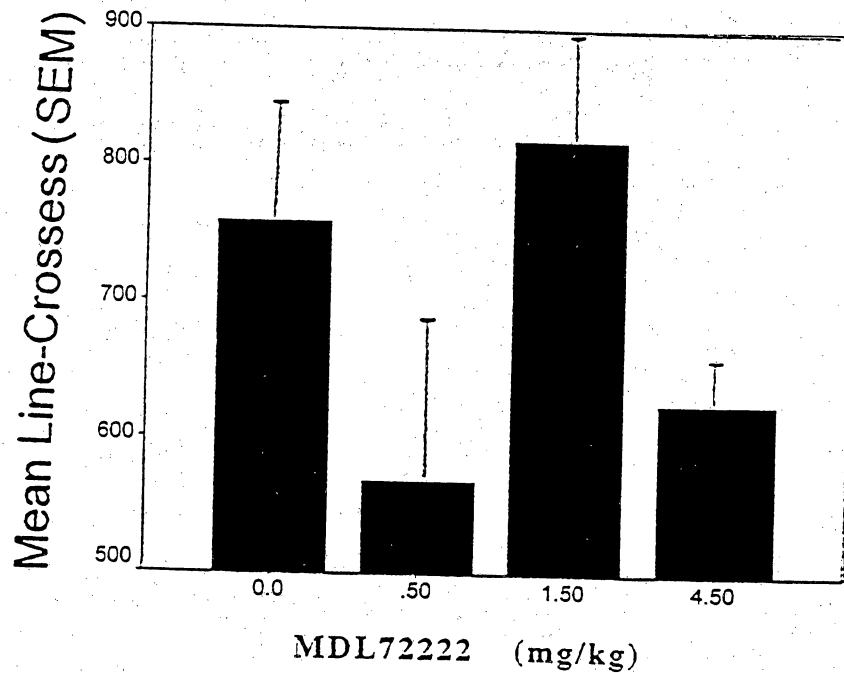


Figure 23. Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each). The 17-day-old rat pups were injected with cocaine (20.0 mg/kg, IP), or saline 30 min following a dose of MDL72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) and placed into the conditioning chamber (n=7).

## LOCOMOTOR ACTIVITY

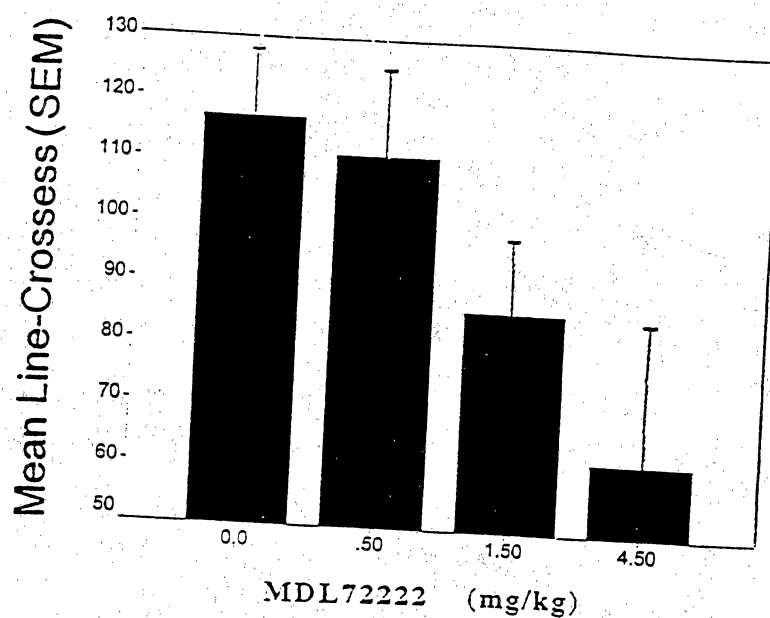


Figure 24. Mean number (+SEM) of line-crosses in the drug-paired compartment of the CPP apparatus during both conditioning sessions (30 min each). The 17-day-old rat pups were injected with saline 30 min following a dose of MDL72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) and placed into the conditioning chamber (n=7).



# EFFECT OF MDL-72222 ON THE ACQUISITION OF MORPHINE CPP

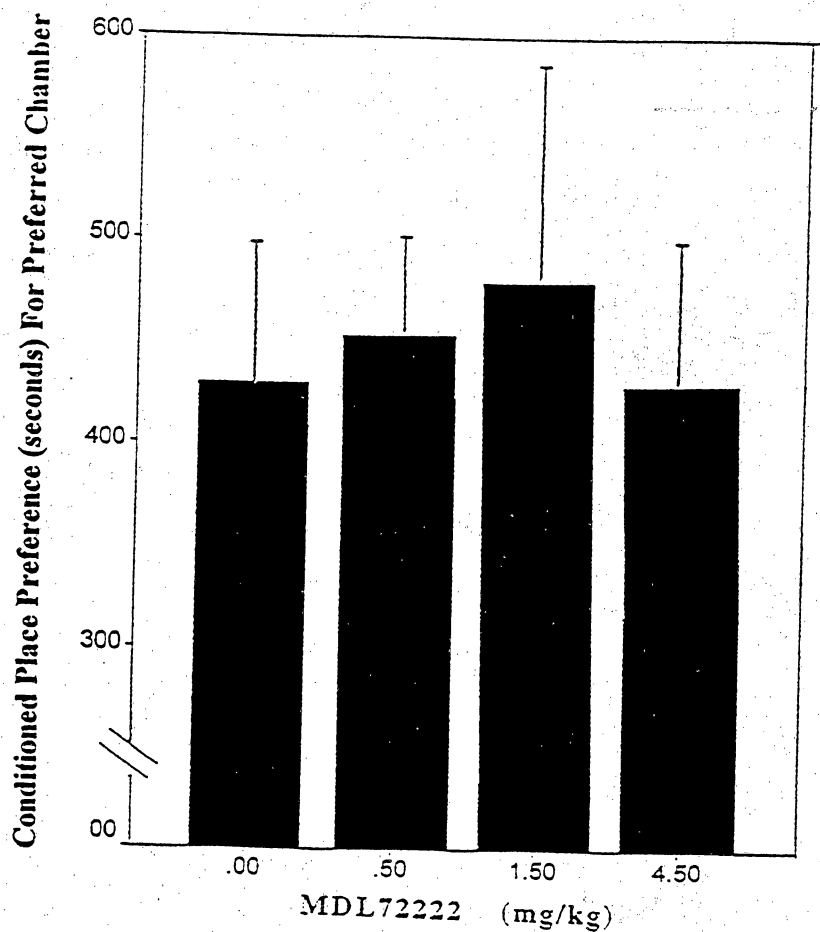


Figure 25. Mean time that rats spent (seconds) in the morphine-paired chamber (+SEM) of the CPP apparatus on test day. On the previous day, each 17-day-old rat pup was given MDL72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) 30 min prior to an injection of saline or morphine (0.5 mg/kg, IP) and allowed 900 sec access to the three compartments (n=7).

# EFFECT OF MDL-72222 ON THE ACQUISITION OF COCAINE CPP

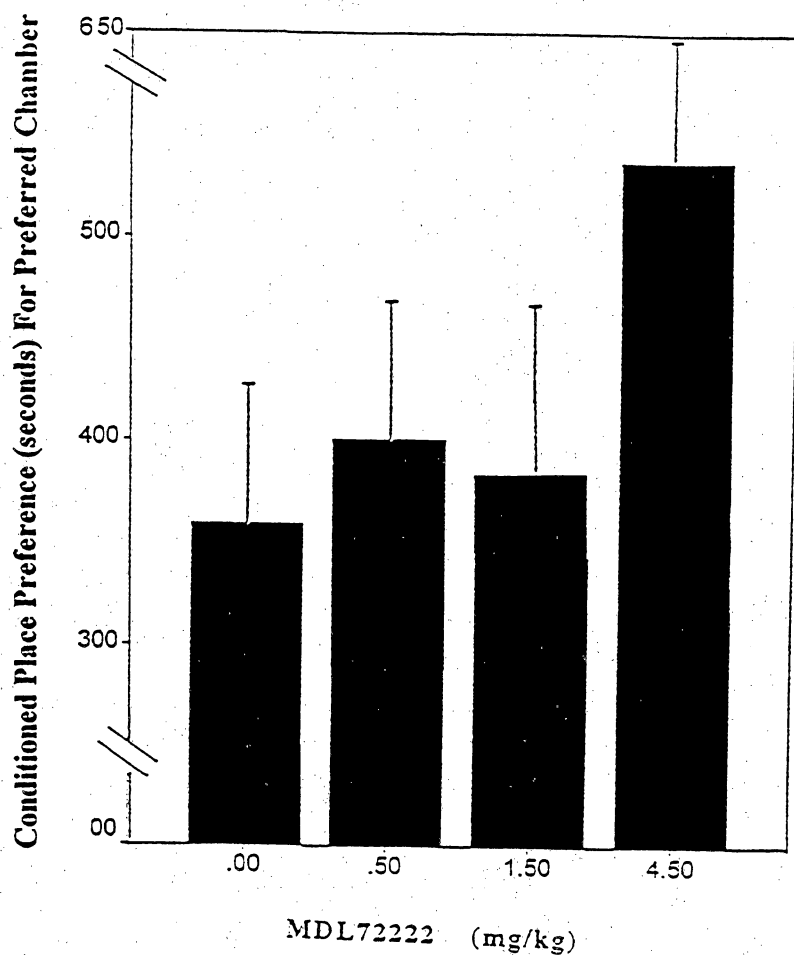


Figure 26. Mean time that rats spent (seconds) in the cocaine-paired chamber (+SEM) of the CPP apparatus on test day. On the previous day, each 17-day-old rat pup was given MDL72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) 30 min prior to an injection of saline or cocaine (20.0 mg/kg, IP), and allowed 900 sec access to the three compartments (n=7).

Treatment with MDL-72222 did not affect compartment preference in saline-conditioned rats,  $F(3,24) = .85$ ,  $P > .05$  (see Figure 27).

On the test day, rat pups showed only a nonsignificant preference for the morphine- or cocaine-paired chamber,  $t(12) = 1.12$ ,  $P > .05$ ;  $t(12) = .55$ ,  $P > .05$  respectively (see Figures 25 and 26).

### Summary

MDL-72222, at dosage levels of 0.5 to 4.5 mg/kg, did not significantly affect the acquisition of morphine- or cocaine-induced CPP. In contrast to Experiment 1, MDL-72222 did not affect compartment preference or action based on cocaine-induced locomotor activity.

# EFFECT OF MDL-72222 ON THE ACQUISITION OF A PLACE PREFERENCE

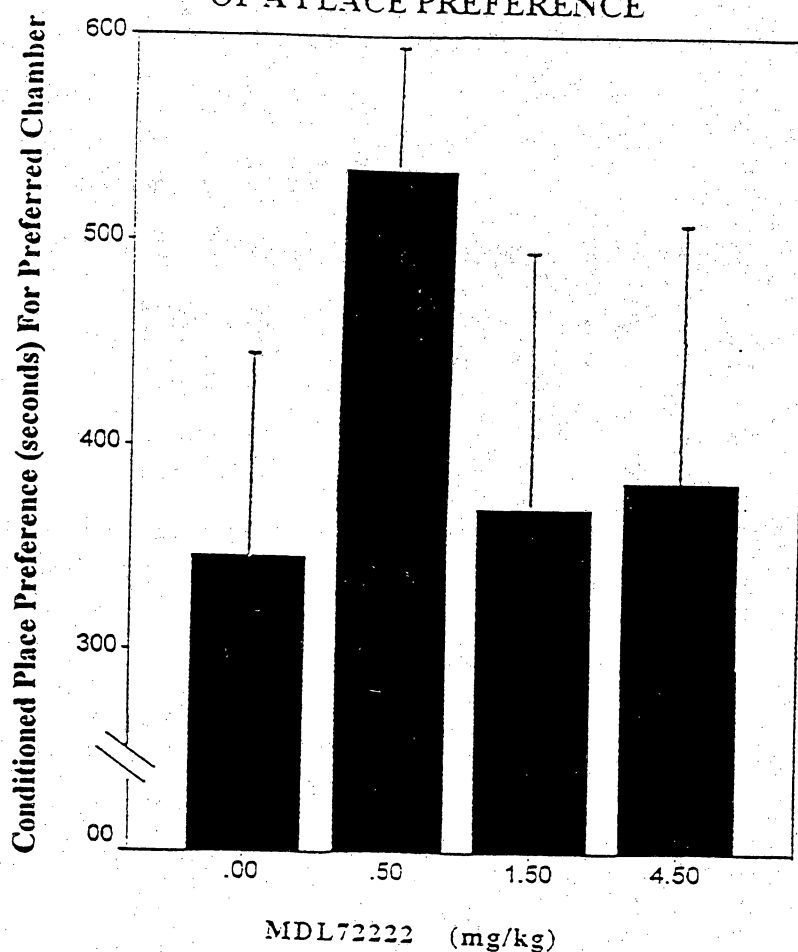


Figure 27. Mean time that rats spent (seconds) in the saline-paired chamber (+SEM) of the CPP apparatus on test day. On the previous day, each 17-day-old rat pup was given MDL72222 (0.0, 0.5, 1.5, 4.5 mg/kg, IP) 30 min prior to an injection of saline and allowed 900 sec access to the three compartments (n=7).

## DISCUSSION

Although there is much evidence to show that the dopamine system in the adult rat is modulated by 5-HT<sub>3</sub> receptors (Higgins et al., 1992; Imperato & Angelucci, 1989; Pei et al., 1989; Suzuki et al., 1993), this is the first study to examine the role, if any, played by 5-HT<sub>3</sub> receptors on the reward processes of the preweanling rat. The results of the present study suggest that the 5-HT<sub>3</sub> receptor system may differentially modulate DA- and opioid-mediated reward across ontogeny. More specifically, 5-HT<sub>3</sub> receptor antagonists block the cocaine- and morphine-induced CPP of adult rats, while a 5-HT<sub>3</sub> antagonist did not block CPP in preweanling rats. There are two probable explanations for these results. First, the 5-HT<sub>3</sub> receptors of preweanling rats may not modulate DA reward in the same manner as in the adult rat. This has not been adequately examined, although it is known that 5-HT<sub>3</sub> receptors are present and functional shortly after birth (Boess & Martin, 1994; Daval et al., 1987; Hard & Engel, 1988; Johansson-Wallsten et al., 1993; Pranzatelli et al., 1992; 1994). Second, 5-HT<sub>3</sub> antagonists may differentially affect the D<sub>1</sub> or D<sub>2</sub> receptors of preweanling rats. Previous research indicates that the DA receptor subtypes of preweanling rats differ in density and expression from those found in adult rats

(McDougall et al., 1991; 1992; Murrin, Gibbens, & Ferrer, 1985; Murrin & Zeng, 1989; 1990; Zeng et al., 1988).

Importantly, the present results demonstrate that both morphine- and cocaine-treated 17-day-old rats showed a robust place preference using the abbreviated CPP procedure. Surprisingly, this study also clearly demonstrates the inability of MDL-72222 (0.17, 0.5, 1.5, 4.5 mg/kg, IP) to significantly block either the expression or acquisition of cocaine- and morphine-induced CPP in 17-day-old rats. This differs from previous studies using adult rats which have shown that 5-HT<sub>3</sub> antagonists block the initial acquisition of a rewarded behavior (Bilsky & Reid, 1991; Carboni et al., 1989; Higgins et al., 1992). More specifically, many 5-HT<sub>3</sub> antagonists, including MDL-72222, have been repeatedly used to attenuate the acquisition of reward, including morphine- and cocaine-induced CPP (Blandina et al., 1983; 1989; Costall et al., 1987; Dyr & Kostowski, 1995; Gifford & Wang, 1994; Grant, 1995). The inability of MDL-72222 to affect CPP in the present study was particularly unexpected because research has suggested that serotonin levels and 5-HT receptor subtypes are present and/or functional shortly after birth (Boess & Martin, 1994; Daval et al., 1987; Hard & Engel, 1988; Johansson-Wallsten et al., 1993; Pranzatelli et al., 1992; 1994). Significantly, research into dopamine receptor functioning across ontogeny has

demonstrated that differences exist between adult and pre-adult responses within the dopamine system (McDougall et al., 1991; 1992; Murrin et al., 1985; Murrin & Zeng, 1989; 1990; Zeng et al., 1988).

Additionally, it was unexpectedly discovered that saline-treated rats given 4.5 mg/kg MDL-72222 on the test day had a significant preference for the compartment used on the previous conditioning day (see Figure 18). This was probably a spurious result since MDL-72222 did not induce CPP when given during conditioning (see Figure 27).

The release of dopamine is believed to be essential for associative learning to take place during rewarding experiences (Wickens, Begg, & Arbuthnott, 1996). However, there has been some disagreement pertaining to the differential involvement of D<sub>1</sub> and D<sub>2</sub> receptors in the reward processes of both adult and young rats. Some research using adult rats has suggested that both the D<sub>1</sub> and D<sub>2</sub> receptors are essential for the acquisition of reward (Miller et al., 1990; Nakajima & Baker, 1989; Nakajima & McKenzie, 1986; Wise & Rompre, 1989). In contrast, Shippenberg et al. (1993) have demonstrated that the rewarding effects of opioids, such as morphine, are primarily dependent upon activation of D<sub>1</sub> post-synaptic receptors located within the NA. Similarly, using adult rats, Hiroi and White (1991a) have demonstrated a differential involvement of dopamine D<sub>1</sub> and D<sub>2</sub> receptor subtypes during the

expression and acquisition of dopamine-induced agonist reward. Hiroi and White (1991a) found that D<sub>2</sub> receptor antagonism failed to block the expression of amphetamine-induced CPP. However, expression of a previously established CPP was blocked by a post-conditioning treatment of a D<sub>1</sub> antagonist. They also found that the expression of reward by D<sub>1</sub> receptors takes place within the NA, which has connections to the amygdala.

The different roles played by D<sub>1</sub> or D<sub>2</sub> dopamine receptors in the preweanling rat may explain why 5-HT<sub>3</sub> antagonists did not affect reward processes. Quantitative autoradiographic assessment of dopamine D<sub>1</sub> and D<sub>2</sub> receptors has shown that these DA receptor subtypes differ in their quantity and impact on the reward system during various developmental stages (McDougall et al., 1991; 1992; Murrin, Gibbens, & Ferrer, 1985; Murrin & Zeng, 1986; 1989; 1990; Zeng et al., 1988) (see Figures 28 and 29). Importantly, preweanlings rats exhibit regional brain differences in receptor density when compared to other developmental time periods. For example, preweanling rats possess a greater number of D<sub>2</sub> receptors in the NA, as compared to other forebrain regions (Broadbuss & Bennett, 1990; DeVries, Mulder & Schoffelmeer, 1992; Gelbard et al., 1989; McDougall et al., 1991; Murrin & Zeng, 1989; 1990; Zeng, Hyttel & Murrin, 1988). Additionally, McDougall et al. (1991)



found that 17-day-old rats not only have a higher density of D<sub>2</sub> receptors in the NA, but that they respond differently to D<sub>2</sub> antagonism when compared to other age levels. Pruitt et al. (1995) also found a differential rate of receptor functioning and reward responsiveness across ontogeny in a cocaine CPP study assessing the antagonism of reward by D<sub>1</sub> and D<sub>2</sub> receptor blockade. Importantly, they showed that D<sub>2</sub> antagonists did not block cocaine-induced CPP in 17-day-old rats. It was concluded that D<sub>1</sub> receptors are responsible for mediating reward in this age group.

Preweanling rats show a D<sub>1</sub> and D<sub>2</sub> dopamine receptor ratio of 1:2 in the NA. In contrast, D<sub>2</sub> receptor levels maintain a steady increase during development, whereas D<sub>1</sub> receptors show a sudden increase, peaking just before and following weaning (Gelbard et al., 1989; Murrin & Zeng, 1990). Using D<sub>1</sub> and D<sub>2</sub> receptor antagonists to block CPP at various ages, Pruitt et al. (1995) concluded that D<sub>1</sub> receptors are critically involved in the reward processes of preweanling rats, especially during this peak increase. Specifically, when D<sub>1</sub> receptor density is low, as it is in adult and 10-day-old rats, D<sub>2</sub> receptor blockade appears to be more effective in antagonizing DA reward. Nonetheless, it has been concluded that D<sub>1</sub> and D<sub>2</sub> receptors may be coupled in reward processes (Pruitt et al., 1995). Thus, each receptor subtype cooperates in an

interactive manner to provide tonic motor ( $D_2$ ) and reward sensations ( $D_1$ ) that are necessary for the full expression of the reward system (see also Wickens et al., 1996). This may explain the findings in the current study, which showed that 5-HT<sub>3</sub> receptor antagonism failed to affect DA reward in preweanling rats. The sudden increased peak affect of the  $D_1$  receptor just prior to and following weaning may have prevented the blockade of reward by the 5-HT<sub>3</sub> antagonist (see Figures 28 and 29).

Additionally, the role played by memory in conditioned learning is currently being reduced to the level of receptors, as researchers attempt to determine the importance of receptor subtypes for the establishment and retrieval of memory (Desimone, 1995). Dopamine receptors have been implicated in short-term memory and NMDA and AMPA receptors appear to mediate stimulus-affect associations, whereby an animal recognizes and evaluates the biological significance of the stimulus (Ono et al., 1995). The retention of the memory of a rewarding experience is believed to be processed within the amygdala (Hiroi & White 1991a; Ono et al., 1995). This brain structure has a high density of  $D_1$  receptors (Hiroi & White, 1991a; 1991b), and the globus pallidus which connects the NA to the amygdala, is also high in  $D_1$  receptors (Murrin & Zeng, 1990).

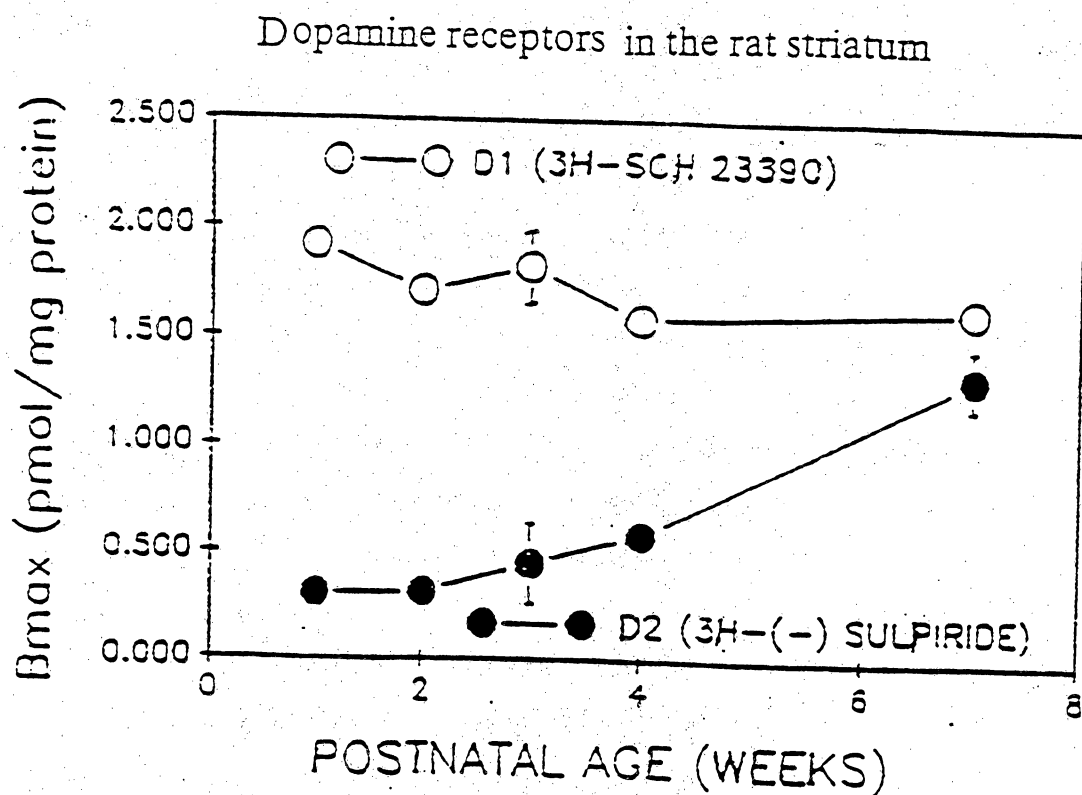


Figure 28. Development of D<sub>1</sub> and D<sub>2</sub> receptor sites through equilibrium saturation studies of D<sub>1</sub> (<sup>3</sup>H-SCH 23390) and D<sub>2</sub> (<sup>3</sup>H]-(-) sulpiride) binding at increasing ages in rat brain development. (Copied from Giorgi et al., 1987).

## Regional Developmental Differences in D1 Density

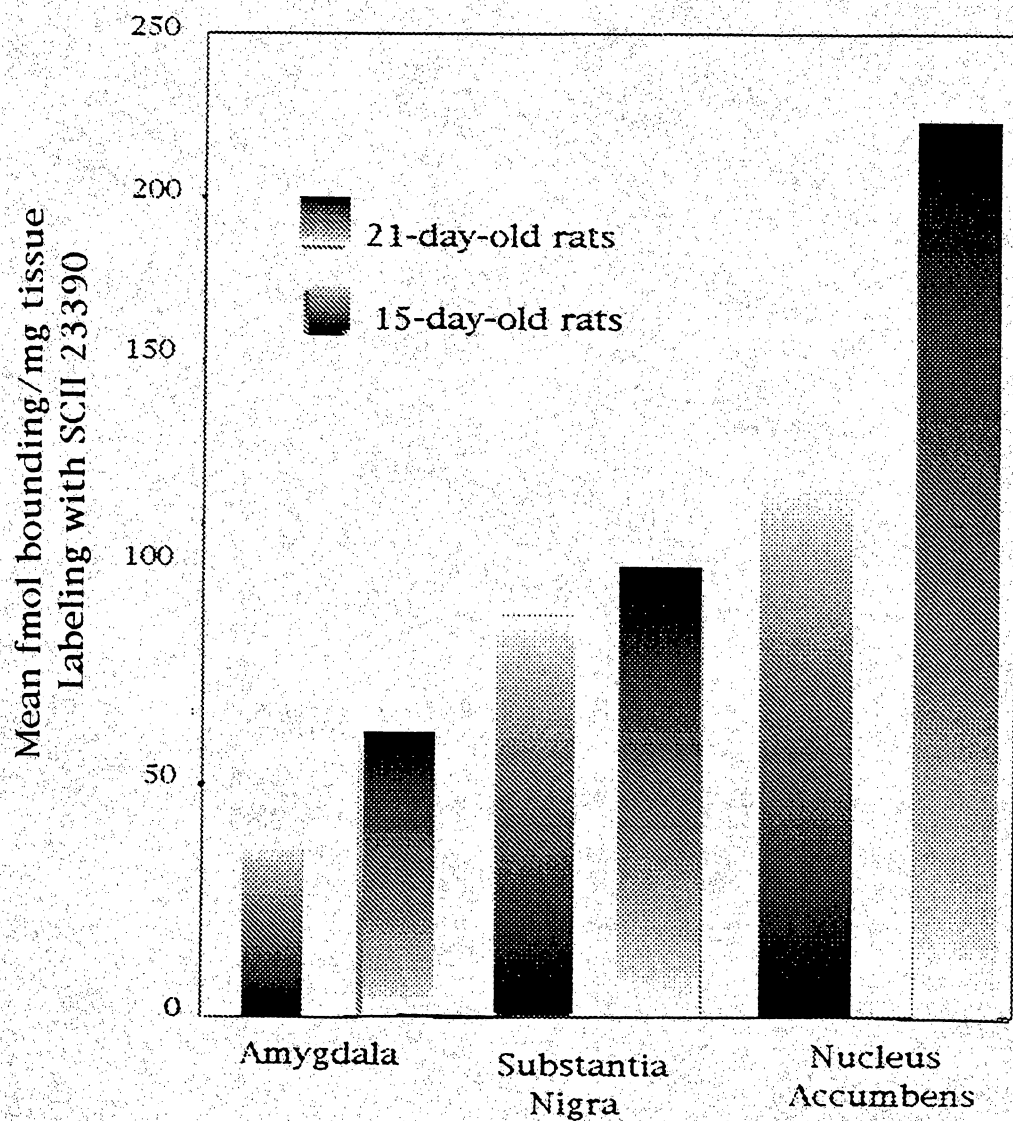


Figure 29. Mean regional density of D1 receptors during development of the 15-day-old to 21-day-old (Adapted from Murrin & Zeng, 1990).

The differential development of dopamine receptor subtypes have also been found to occur in humans and primates (Hyttel & Arnt, 1986; Williams & Goldman-Rakic, 1995). Specifically, dopamine D<sub>1</sub> receptors have been correlated with certain types of memory and children demonstrate peak densities of these receptors during developmental stages when learning has been shown to be especially accelerated (Desimone, 1995; Williams & Goldman-Rakic, 1995).

Along with a developmental increase in memory capacity in children ages 4 to 10 years old, there is a concomitant and temporary excess of D<sub>1</sub>, as compared to D<sub>2</sub>, dopamine receptors at this mentally accelerated age-range in humans (Hyttel & Arnt, 1986).

As mentioned before, the D<sub>1</sub> dopamine receptor sites of rats increase dramatically in number on those days just prior to, and following, the weaning period; whereas, D<sub>2</sub> receptor density (believed to be important for activity) is greater within the striatum during this time (Broaddus & Bennett, 1990; DeVries et al., 1992; Gelbard et al., 1989; Murrin & Zeng, 1986; 1990) (see Figure 28). Although speculative, this may relate to an increased need for the experience of reward, the retention of its memory, and its retrieval, possibly through the activation of increased D<sub>1</sub> receptors, located within the amygdala and surrounding regions (Hiroi & White, 1991b) (see Figure 30).

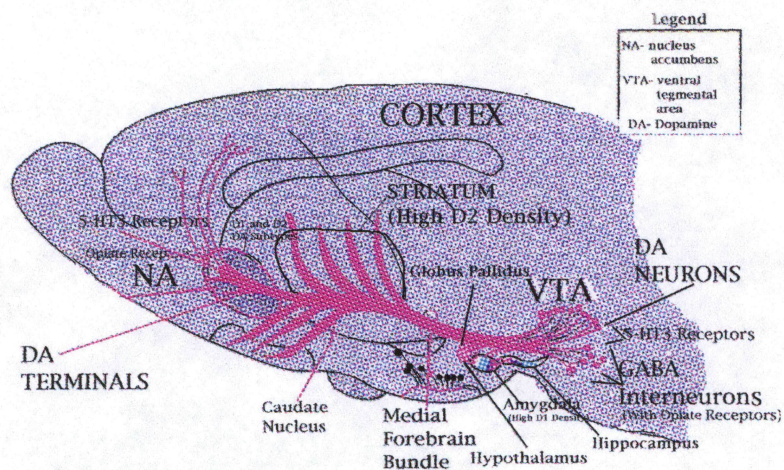


Figure 30. Dopamine receptors D1 and D2 in rat brain.

The amygdala is believed to be involved in emotionally charged mnemonic processes, playing a large role in conditioned associative learning (Hiroi & White, 1991b; Ono et al., 1995). A rat pup that is nearing the weaning stage needs to more readily retrieve the previously learned connections between rewarding experiences and associated stimuli; the memory of how that reward was obtained. Its' survival depends upon an increased ability to learn, and to retrieve these memories in the absence of the nurturing mother.

The increase in D<sub>1</sub> receptors at this critical developmental period provides a plausible explanation for the inability of 5-HT<sub>3</sub> antagonists to suppress the expression and acquisition of reward in preweanling rats. Receptors capable of suppressing reward at this precarious developmental period must necessarily be tempered.

## CONCLUSION

The results of the present study indicate that 5-HT<sub>3</sub> receptor functioning differs between preweanling and adult rats. While 5-HT<sub>3</sub> receptors modulate reward processes of adult rats, they appear to have little effect on the reward functioning of preweanling rats. This may be due to the increased importance of D<sub>1</sub> receptor functioning at this critical stage of life.

The differential involvement of D<sub>1</sub> and D<sub>2</sub> receptors in the expression and acquisition of reward processes in adults, implies that D<sub>2</sub> receptors have an increased importance in the acquisition of reward, while D<sub>1</sub> receptors function in the expression of reward (Hiroi & White, 1991a; 1991b). The receptors involved in memory processes that occur in relation to the acquisition and expression of conditioned learning is an important area for further research.

Until the present study, there have been no studies examining the role of 5-HT<sub>3</sub> receptors for the expression or acquisition of reward in preweanling rats. Our findings demonstrate the inability of the specific 5-HT<sub>3</sub> antagonist MDL-72222 to block the expression and acquisition of morphine- and cocaine-induced reward and I believe that this may be related to the ontogenetic peak of D<sub>1</sub> receptors which occurs in the



preweanling rat (Broadbuss & Bennett, 1990; DeVries, Mulder, Schoffelmeer, 1992; Hiroi & White, 1991a; 1991b; Murrin & Zeng, 1986;1990; Pruitt et al., 1992).

However, it is important to replicate these results by using different experimental designs. Our finding that 5-HT<sub>3</sub> antagonism fails to attenuate preweanling DA reward, obviously differs from the adult rat suggesting many other possibilities. The preweanling rat may possess 5-HT<sub>3</sub> receptors that are too immature to respond to the antagonist at the dosage levels tested. Similarly, 5-HT<sub>3</sub> antagonism using the abbreviated CPP paradigm, should be repeated in adult rats in order to rule out any spurious differences found in this current study. Possibly, a different paradigm such as the standard CPP procedure or the operant conditioning paradigm could produce differing results. Similarly, the injection of different levels of 5-HT<sub>3</sub> antagonist drug may produce a different response in the preweanling rat.

Further research is needed to identify the mechanisms involved in serotonin modulation of reward in this age group. However, it remains that the inability of MDL-72222 to block expression of CPP may not be generalizable to adult rats. This is particularly likely, since various 5-HT<sub>3</sub> antagonists, including MDL-72222, block the acquisition of reward in adult rats (Blandina et al., 1983; 1989; Costall et al., 1987; Dyr &

Kostowski, 1995; Gifford & Wang, 1994; Grant, 1995; Higgins et al., 1992; Imperato & Angelucci, 1989).

In contrast to adult rats, the present study indicates that 5-HT<sub>3</sub> antagonists may be incapable of blocking the acquisition or expression of reward in preweanling rats. I believe that the peak occurrence of D<sub>1</sub> receptors at this period of development is related to the inability of the 5-HT<sub>3</sub> receptor antagonist to block morphine- and cocaine-induced reward. The temporary surge of D<sub>1</sub> receptors in preweanling rats, as compared to D<sub>2</sub> receptors, may have developed as a survival trait that acts to enhance the retrieval of conditioned associations for the memory of the sensation and the source of the reward. This is the time in rat development when the ability to form these associations is particularly important for the survival of the animal, as maternal nourishment is withheld. Survival of the individual depends upon learning the associations necessary for successful foraging behavior. Reward is an integral aspect of this learning.

Further research is needed to confirm the specific effects that 5-HT<sub>3</sub> receptor antagonists have on D<sub>1</sub> receptors, and the specific role, if any, that 5-HT<sub>3</sub> receptors have in the reward processes of preweanling rats. The current study confirms that 5-HT<sub>3</sub> receptors differentially affect the acquisition and expression of DA reward across ontogeny.

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